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COMPREHENSIVE INVITED REVIEW

Redox Regulation of DNA Repair: Implications for Human Health and Cancer Therapeutic Development

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Abstract

Redox reactions are known to regulate many important cellular processes. In this review, we focus on the role of redox regulation in DNA repair both in direct regulation of specific DNA repair proteins as well as indirect transcriptional regulation. A key player in the redox regulation of DNA repair is the base excision repair enzyme apurinic/apyrimidinic endonuclease 1 (APE1) in its role as a redox factor. APE1 is reduced by the general redox factor thioredoxin, and in turn reduces several important transcription factors that regulate expression of DNA repair proteins. Finally, we consider the potential for chemotherapeutic development through the modulation of APE1's redox activity and its impact on DNA repair. *Antioxid. Redox Signal.* 12, 1247–1269.

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I. Introduction

ALTHOUGH THE IMPORTANCE of DNA-repair pathways in protecting the genome from damage caused by endogenous and exogenous DNA-damaging agents (40, 44, 60) has long been recognized, the role of redox regulation in these pathways is a relatively recent discovery. In writing this review, we attempted to guide the reader through general as well as specific aspects of DNA repair and redox regulation, focusing ultimately on the connection between the two. We begin with an overview of DNA-repair pathways leading to a more in-depth discussion of one specific DNA-repair pathway, the base excision repair (BER) pathway. We focus on the BER pathway, which is responsible for the repair of DNA damage caused by oxidation, alkylation, and ionizing radiation, and specifically on apurinic/apyrimidinic endonuclease 1 (APE1), the only DNA-repair protein currently known to serve a dual role as a repair enzyme and a redox factor. In its role as a redox factor, APE1 modifies downstream transcription factors such as AP-1, NF-κB, CREB, p53, and others, and thereby indirectly alters the activity of other DNA-repair pathways. To put the redox activity of APE1 in perspective, we provide an overview of general redox systems as well as an in-depth discussion of the redox activity of APE1. Finally, in considering the impact of redox regulation of DNA repair to human health, we discuss the modulation of the redox activity of APE1 by small molecules and the potential for chemotherapeutic development targeting redox regulation of DNA repair.

II. DNA-Repair Pathways

The genome of eukaryotic cells is constantly under attack from both endogenous and exogenous DNA-damaging agents. DNA damage resulting from endogenous agents includes oxidation by reactive oxygen species (ROS) generated from normal metabolic processes, alkylation by agents such as *S*-adenosylmethionine, adduct formation resulting from attack by reactive carbonyl species formed during lipid peroxidation, hydrolytic depurination leading to the formation of abasic sites, or deamination of bases, primarily cytidine, and to a lesser extent, adenine (44). Exogenous agents include environmental insults (chemicals, carcinogens, UV light), chemotherapeutic agents, and radiation damage (40, 60). Failure to repair DNA damage in both postmitotic and mitotic

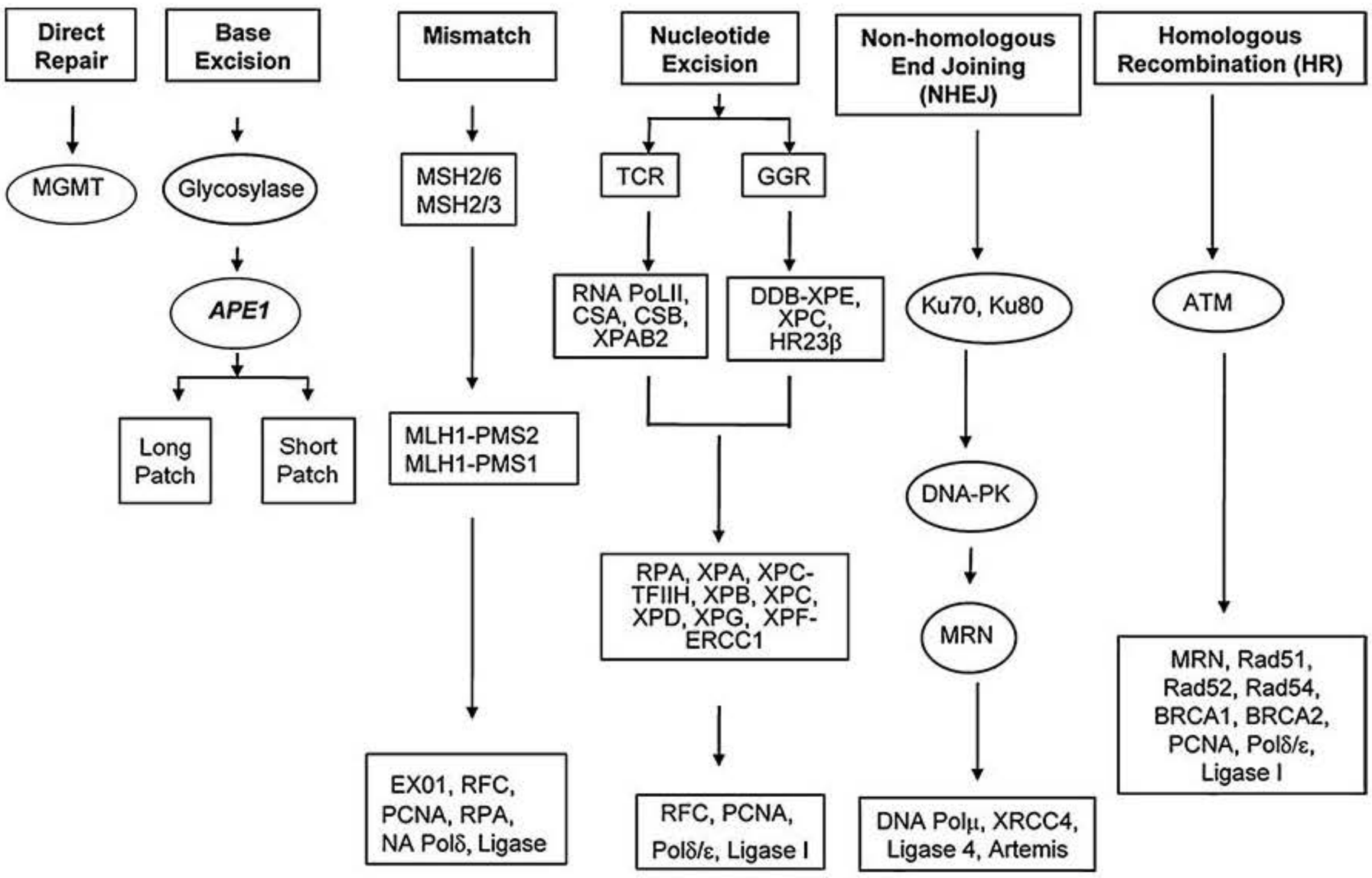

FIG. 1. Schematic overview of DNA-repair pathway. Several DNA-repair pathways are involved in maintaining cell genomic stability; these include direct repair (DR), base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), homologous recombination (HR), and nonhomologous end joining (NHEJ). More than 150 proteins are involved. Only selected genes of each pathway are shown here. [Adapted from Fishel *et al.* (57).]

cells can result in apoptosis or accumulation of mutations and even cell-cycle arrest (58, 106). For example, the DNA-damage response in mitotic cells results in cell-cycle arrest involving the major cell-cycle machinery. In postmitotic cells, DNA damage may result in cell-cycle activation and subsequent arrest, leading to deleterious events in this cell population as well (110, 160). However, we have evolved a series of DNA-repair pathways to correct the damage, including direct repair (DR), base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), homologous recombination (HR), and nonhomologous end joining (NHEJ) (85, 86) (Fig. 1). The number of DNA-repair proteins and factors involved in the cellular response to DNA damage keeps growing as more and more information is obtained, not only on the DNA repair enzymes involved in each pathway, but also on the regulatory networks that are induced by persistence of DNA damage in the cell (182). Distinct DNA damage is repaired by the different pathways and mechanisms. Overlap and interaction between the various pathways and some overlap in mechanisms occur. For example, O^6-methylguanine can be removed directly by O^6-methylguanine-DNA methyltransferase (MGMT or AGT) in DR, but if this pathway is not successful, the O^6mG mispairs and is recognized by the MMR pathway (59). Similarly, oxidative DNA damage is repaired mainly by BER, but some repair by NER also is possible (53). Single-strand DNA breaks (SSBs) unrepaired by BER lead to double-strand breaks (DSBs), which may be repaired by HR, and HR can also repair DNA DSBs that NHEJ pathways fail to process (49). Interaction of different DNA-repair pathways and mechanisms provides the most efficient defense for the cell genome, whereas reduced repair capacity can lead to genomic instability. A number of diseases are associated with defects in DNA repair, including xeroderma pigmentosum, Cockayne syndrome, tridothiodystrophy, Werner syndrome, and Bloom syndrome (118, 162). The reader is directed to recent comprehensive reviews for more specific information on each DNA-repair pathway (47, 111, 144, 145, 150, 157, 174). For updated information on the individual repair proteins, the following link may prove useful: http://www.cgal.icnet/DNA_Repair_Genes.html (182). What follows is an overview of DNA-repair pathways necessary to provide a context for understanding the role of redox regulation in DNA repair.

A. Mammalian direct repair: O^6-alkylguanine-DNA methyltransferase or O^6-methylguanine-DNA methyltransferase

This type of repair in mammals is termed direct reversal because the damaged base is repaired through removal of the alteration to the base instead of removal of the damaged base. It is unique in this sense and probably is the most efficient mechanism of repair (105). The protein that carries out this reaction, the AGT protein, removes alkyl groups through direct transfer from the O^6 position of guanine and to a lesser extent from the O^4 position from thymine to the protein, leaving a guanine or thymine in DNA and inactivated protein. This is a stoichiometric reaction, as one protein removes one alkyl group and is then degraded. It is essential to repair O^6-meG adducts, as they cause errors by mispairing with thymine during replication, leading to G:C to A:T transitions or a strand break.

B. Base-excision repair

BER is responsible for the repair of DNA damage arising from alkylation, deamination, or oxidation of bases (8, 40, 50). Alkylation of bases arises from exposure to either endogenous agents such as *S*-adenosylmethionine or exogenous agents, including environmental and chemotherapeutic agents, whereas deamination of cytidines and adenines occurs spontaneously. Oxidative damage can result from ROS generated by normal cellular processes, in addition to environmental or chemotherapeutic agents. BER is initiated by the removal of the damaged base through enzymes called DNA glycosylases, which specifically recognize several different types of base damage. Glycosylases are of two types, monofunctional and bifunctional. Monofunctional glycosylases [*e.g.*, *N*-methyl purine DNA glycosylase (MPG or AAG)] excise the damaged base to generate an apurinic/apyrimidinic (AP) or abasic site, which is acted on by the multifunctional AP endonuclease, APE1. Bifunctional glycosylases such as human 8-oxoguanine DNA glycosylase (hOGG1), human endonuclease VIII–like DNA glycosylase (NEIL1-3), and *E. coli* endonuclease III (NTH) glycosylase have an additional AP lyase function (36, 43) that excises the damaged base and nicks the phosphodiester backbone 3′ to the AP site. The resulting AP site is processed by APE1, which hydrolyzes the phosphodiester backbone immediately 5′ to the AP site, creating 3′ OH and 5′ deoxyribose phosphate (5′ dRP) termini. At this stage, repair can proceed by two pathways: the short-patch BER (SP-BER) pathway and the long-patch BER (LP-BER) pathway. APE1 is responsible for 95% of the endonuclease activity in the cell and is a critical part of both the short-patch and the long-patch BER pathway (45, 46). SP-BER repairs normal AP sites. DNA polymerase β (pol β) removes the 5′ dRP moiety by its dRPase activity and uses the 3′ OH terminus to insert the correct base. The nick is ligated by DNA ligase III/XRCC1, and repair is completed. The LP-BER pathway preferentially repairs oxidized and reduced AP sites and is a minor branch of the BER pathway. A segment or flap of three to eight nucleotides surrounding the AP site is displaced, followed by insertion of the correct nucleotides by DNA polymerase δ, ε, or β, along with proliferating cell nuclear antigen (PCNA) and replication factor-C (RF-C). After resynthesis, flap endonuclease 1 (FEN1) removes the displaced strand, and DNA ligase I, or the DNA ligase III/XRCC1 complex ligates the nick. APE1 is the only AP endonuclease that performs these functions in the BER, and as such, is a key player in the BER process. APE1 also coordinates recruitment of other DNA-repair proteins involved in BER through a complex network of direct protein–protein interactions and indirect interactions, as shown in Fig. 2. No effective backup to APE1 activity exists in the cell, as is discussed in more detail later, including its other major redox-signaling function.

C. Nucleotide-excision repair

The NER pathway is responsible for repairing large adducts such as ultraviolet-light–induced cyclobutane pyrimidine dimers, adducts induced by polycyclic aromatic hydrocarbons, and other bulky DNA lesions induced by cross-linking agents and base-damaging chemical carcinogens. Numerous proteins are required to complete NER. More than 25 proteins/complexes have been identified in eukaryotic cells; these can be further divided into two main

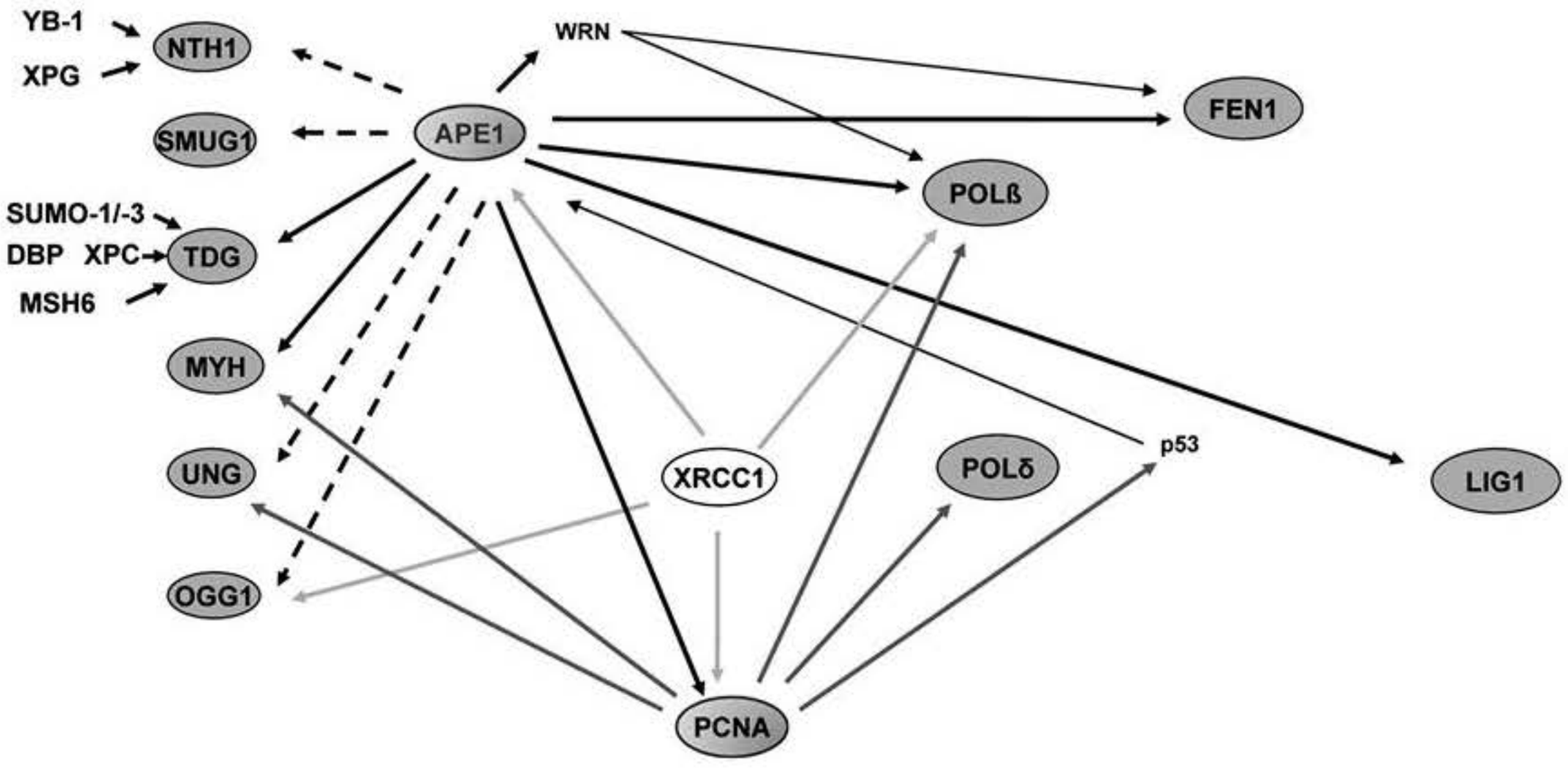

FIG. 2. Network of protein–protein interactions involving APE1 (adapted from ref. 51). APE1 plays an essential role in BER through its enzymatic activity as well as its role in coordinating interactions, either directly (*solid lines*) or indirectly (*dashed lines*), with a large number of other proteins involved in BER. Proteins highlighted are central components with APE1, apurinic/apyrimidinic endonuclease, in the middle. Those proteins in the first column are DNA glycosylases; the remaining proteins include PCNA, an accessory protein; polymerases, pol β and pol δ; ligase 1, LIG1; and the flap endonuclease, FEN1.

subpathways; global genome repair (GGR) and transcription-coupled repair (TCR), depending on the complexes that initiate repair (8, 63). TCR is initiated when RNA polymerase II (RNA Pol II) stalls at sites of DNA damage. TCR-specific factors, including the Cockayne syndrome proteins, CSA and CSB, are recruited at the site of transcription arrest, followed by removal of the lesion by NER enzymes. In contrast, the heterodimer XPC/HR23B appears to be the major damage-recognition factor in human cells. The UV-DNA damage–binding protein UV-DDB is additionally required for NER of UV-induced cyclobutane pyrimidine dimers. After recognition, both TCR and GGR use the same proteins to repair the damaged DNA. The transcription-factor IIH (TFIIH) complex is recruited to the site of DNA damage, including its component helicases, XPB and XPD (xeroderma pigmentosum complementary group B and D proteins) that unwind the DNA strand on either side of the DNA damage. XPA and RPA (replication protein A) stabilize the exposed single-strand DNA followed by cleavage of the 27- to 30-nucleotide fragment 3′ and 5′ of the lesion by endonucleases XPG and ERCC1/XPF1/XPF. The resulting gap is filled in by the DNA polymerases δ or ε, along with PCNA, RPA, and replication factor C (RFC) by using the undamaged strand as a template.

D. Mismatch repair

In a broad definition, MMR is responsible for the recognition and repair of single mismatches or misaligned short nucleotide repeats. Mismatches can be endogenously caused by spontaneous deamination of 5-methylcytosine to thymine, resulting in a guanine-to-thymine mismatch, damage to the cellular nucleotide pool, cytosine deamination to uracil, resulting in a guanine-to-uracil mismatch, or incorrect incorporation by DNA polymerase. A complex of MSH2 and MSH6 recognizes the mismatch and initiates the pathway. Various combinations of MSH2 and either MSH3 or MSH6 are formed, which specify the type of mismatch recognized. For example, when MSH2 is paired with MSH6, it recognizes both insertion–deletion mispairs and single-base mismatches, whereas when it is paired with MSH3, the complex recognizes insertion–deletion mispairs. After recognition, MSH proteins recruit MLH1 and its binding partners, post–meiotic-segregation increased-1 protein (PMS1) and PMS2. An exonuclease removes the DNA lesion, a DNA polymerase synthesizes a new strand, and finally, a DNA ligase completes the repair. This has been previously reviewed (104, 126).

E. Nonhomologous DNA end-joining and homologous recombination

NHEJ is the main repair pathway for DSBs in mammalian cells. DNA DSBs may be caused by ionizing radiation (IR), chemotherapeutic drugs, cleavage during V (D) J-recombination, meiotic recombination, or the collapse of replication forks. DSBs are the most severe form of DNA damage and endanger genomic stability by coordinating deletion or translocation or both of chromosomal DNA. Proteins including, but not limited to, Ku 70, Ku 80, DNA ligase IV, and XRCC4 are part of the NHEJ-repair pathway. The Ku proteins bind to the ends of broken DNA and, as a complex with DNA-PK_{cs} (DNA-dependent kinase catalytic subunit), interact with DNA ligase IV and XRCC4 to repair DNA through the NHEJ pathway. DNA ligase IV and XRCC4 function in a complex to ligate the nick and to complete repair (34). Discovery of new proteins involved in NHEJ includes Metnase or SETMAR (117), which has been shown to interact with DNA ligase IV and to enhance the efficiency and accuracy of NHEJ (92).

Homologous recombination (HR) also is involved in repairing DNA DSBs. HR is initiated through the DSB recognition by ATM (ataxia telangiectasia–mutated protein), which phosphorylates multiple downstream proteins. DSBs are processed by the MRN complex (Mre11/Rad50/Nbs1) nuclease activity to yield single-strand DNA (ssDNA). ATM-activated BRCA1 attracts BRCA2 and RAD51 to bind to the

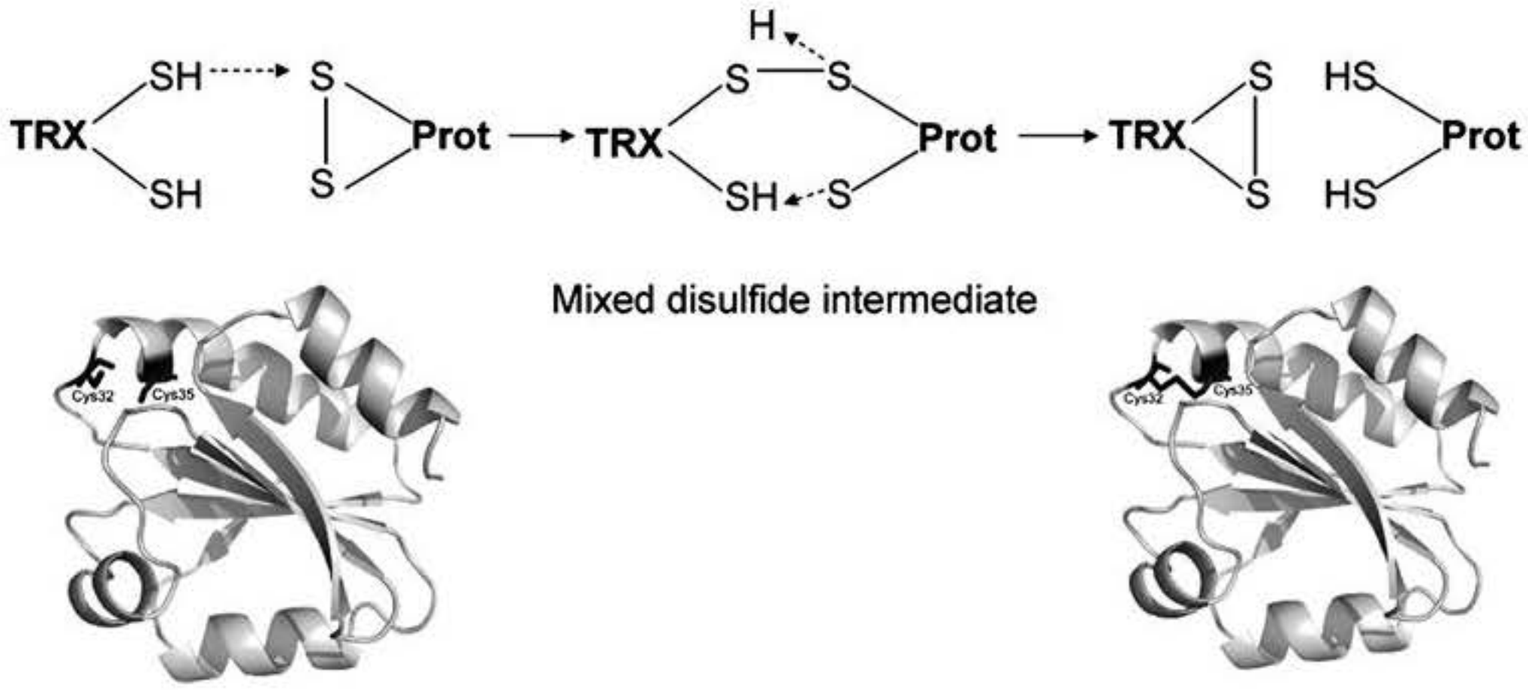

FIG. 3. Reduction of oxidized proteins by TRX. Thioredoxin (TRX) reduces oxidized proteins containing a disulfide through the formation of a mixed disulfide intermediate involving nucleophilic attack by Cys 32 of the CXXC motif. The mixed disulfide is then resolved by Cys 35, resulting in the formation of a disulfide bond in thioredoxin. *Ribbon* renderings are shown for the reduced (PDB identifier, 1ERT) and oxidized thioredoxin (PDB identifier, 1ERU) along with the Cys residues of the CXXC motif in *black-stick* renderings.

ssDNA ends, allowing the RAD52/RAD54 complex to join and form larger complexes with BLM and WRN proteins. These large protein complexes at the strand break direct pairing of the processed DNA with a homologous region on the sister chromatid and initiate strand exchange. This was previously reviewed in detail (167).

III. General Redox Systems

In living systems, two systems are primarily responsible for general reduction-oxidation (redox) regulation, the thioredoxin (TRX) and glutaredoxin/glutathione (GRX/GSH) systems. They maintain the redox cellular homeostasis as well as redox regulate several cellular processes through a thiol–redox mechanism (91, 142). Thiol-based redox mechanisms rely on the special properties of Cys residues, which can adopt 10 different sulfur oxidation states from +6 to −2 (the fully reduced state) (69, 100). Cys can exist in a number of different forms *in vivo*, including cysteinyl radical, sulfenic acid, sulfinic acid, sulfonic acid, cystine, and others [see Jacob *et al.* (100) for a recent review of sulfur chemistry associated with Cys]. Of the types of reactions involving Cys residues, the thiol/disulfide exchange reaction is of relevance to our discussion of general redox systems [reviewed in (69)]. In thiol/disulfide-exchange reactions, an oxidized protein, including a disulfide bond, is recognized by a reduced protein such as TRX or GRX/GSH and is then reduced through the formation of an intermediate mixed disulfide bond (Fig. 3). Cys acts as a nucleophile in this type of reaction (69, 100). Interestingly, TRX and GRX/GSH are reduced by thioredoxin reductase and glutathione reductase, respectively, in a reaction involving both thiol/disulfide exchange and electron-transfer reactions requiring cofactors such as $FADH_2$ and NADPH (Fig. 4) (69, 100)

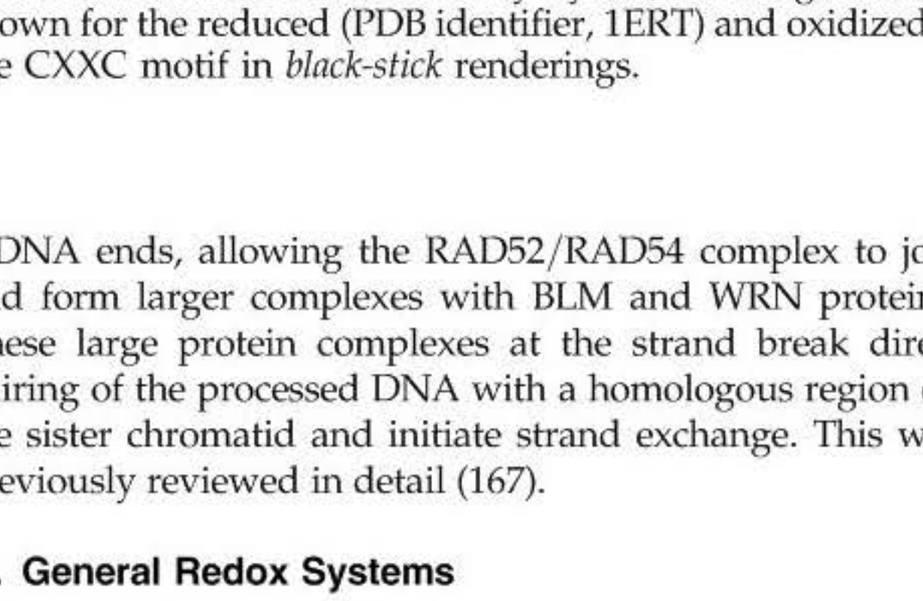

FIG. 4. Thioredoxin reductase/thioredoxin (TR/TRX) redox cascade. Thioredoxin is reduced by thioredoxin reductase in a somewhat more-complex mechanism involving the formation of a selenylsulfide and subsequent reduction by a pair of Cys residues within another subunit of TR. Electron-transfer reactions involving the $FADH_2$, a cofactor of TR, and NADPH are required to regenerate TR. [Adapted from Jacob *et al.* (100).]

A. The thioredoxin system

Components of the thioredoxin system include thioredoxin (TRX), NADPH, and thioredoxin reductase (TR) (90, 100). Thioredoxins (TRXs) comprise a large family of structurally conserved proteins that serve as general protein disulfide oxidoreductases and can reduce disulfide bonds in a variety of proteins through a thiol/disulfide exchange mechanism (143). Oxidized thioredoxin is then reduced by thioredoxin reductase, a flavoprotein containing a selenocysteine, in a reaction involving NADPH.

Thioredoxins (TRXs) share a similar active-site motif Cys-X-X-Cys and a common structural motif, known as the TRX fold (91, 120, 153), which consists of a four-stranded β-sheet surrounded by three α-helices (Fig. 4). The active-site motif is located on the loop connecting β-sheet 1 and α-helix 1. The N-terminal Cys residue in the active site is surface exposed and has a low pK_a value; for example, Cys32 in human TRX has an estimated pK_a of 6.3 (61), whereas the C- terminal Cys is buried in the molecule and has a much higher pK_a value. It has been proposed that the low pK_a value of the N-terminal Cys

arises from the partial positive charge from the dipole moment associated with α-helix 2 (88), or alternatively, may be due to its hydrogen bond to the C-terminal Cys (181). The nucleophilicity of the thiolate group of the Cys is increased by the low pK_a. The proposed reaction mechanism of disulfide reduction by thioredoxin is as follows: the N-terminal cysteine thiolate of TRX acts as a nucleophile and attacks the target disulfide, resulting in a transient mixed disulfide intermediate, which is, in turn, reduced by the C-terminal active-site Cys residue, generating a dithiol in the target protein and a disulfide in thioredoxin (91, 108, 120) (Fig. 3). The resulting disulfide in the active site of TRX can be reduced by TR with electrons from NADPH, completing the catalytic cycle.

The mechanism by which TR reduces TRX back to the dithiol involves the formation of a selenylsulfide in the active site of TR (100), as shown in Fig. 4. A second redox active site located in the other subunit of the dimeric TR contains two thiols that reduce the selenylsulfide back to a thiol and selenol, with the resultant formation of a disulfide bond. This disulfide is reduced by electron transfer from $FADH_2$, and the resulting FAD is then reduced by electron transfer from NADPH (100).

B. The glutaredoxin/glutathione system

The glutaredoxin system is composed of NADPH, the flavoprotein glutathione reductase, glutathione, and glutaredoxin (54, 90, 121). This system also works through a cascade of disulfide oxidation and reduction. Glutaredoxins (GRXs) are small redox enzymes of ~100 amino acid residues, which use glutathione as a cofactor. Structurally glutaredoxins are very similar to thioredoxins, retaining the same fold and active sites. However, the active site of GRXs includes Cys-X-X-Cys or Cys-X-X-Ser. By using a similar reactive mechanism, GRXs catalyze the reversible reduction of substrate protein disulfides, resulting in oxidation of the GRXs (Fig. 5). Oxidized GRXs are reduced nonenzymatically by glutathione (glutamyl-cysteinyl-glycine,GSH), and then the oxidized glutathionine disulfide (GSSG) is reduced by glutathione reductase at the expense of NADPH (121).

C. Roles of general redox systems

Through a thiol/disulfide exchange mechanism, thioredoxin and glutaredoxin systems maintain a reducing intracellular redox state, which is an important metabolic variable, influencing many aspects of cell function, like growth, apoptosis, and reductive biosynthesis. In addition, by redox signaling, they control the activation of a number of transcription factors and hence regulate a broad range of cellular functions (11, 153, 192). The two redox systems physiologically play many roles in different organisms and, conversely, are also pathophysiologic factors for a variety of human diseases, including cancer, viral disease, Alzheimer's disease, and others (7, 12, 33, 154), and hence serve as vital drug targets for cancer therapy and other disease treatments (20, 39, 122, 123, 141). Although the thioredoxin system and glutaredoxin system share a number of functions, they are not just simple duplicate systems; TRX and GRX act on different substrates (54, 121). TRX but not GRX, for example, has been implicated in the reduction of APE1 (6, 83, 94, 170).

The remainder of our discussion on redox factors focuses on APE1, which is the only DNA-repair protein known also to have a role in redox regulation affecting the expression of a number of other DNA-repair proteins.

IV. The Redox Activity of APE1

In a search to identify the nuclear factor responsible for reducing the transcription factor AP-1, a factor termed redox effector factor 1, Ref-1, was identified (184, 187). Since this initial discovery, APE1 (Ref-1) has been reported to reduce a number of other important transcription factors, including NF-κB, HIF-1α, p53, PAX, and others (35, 48, 83, 84, 112, 169, 175) (Fig. 6). And, as discussed below, the redox activity of APE1 plays an important role in regulating the expression of a large number of DNA-repair proteins.

A. Evolution of the redox function of APE1

Although APE1 is reported to have distinct redox and repair domains (186) located within the N- and C-terminal regions of the protein, respectively, these functional domains do not correspond to independently folded domains within the protein (*i.e.*, structural domains). Furthermore, the repair and redox activities do not appear to be coordinated within human APE1, and whereas the AP endonuclease activity of APE1 is conserved from bacteria to humans, the redox function is unique to mammals. Thus, as shown in Fig. 7, APE1 and *E. coli* exonuclease III, the major AP endonuclease found within *E. coli*, are closely related in terms of structure (r.m.s.d., 1.5 Å), retaining not only the same overall fold and topology

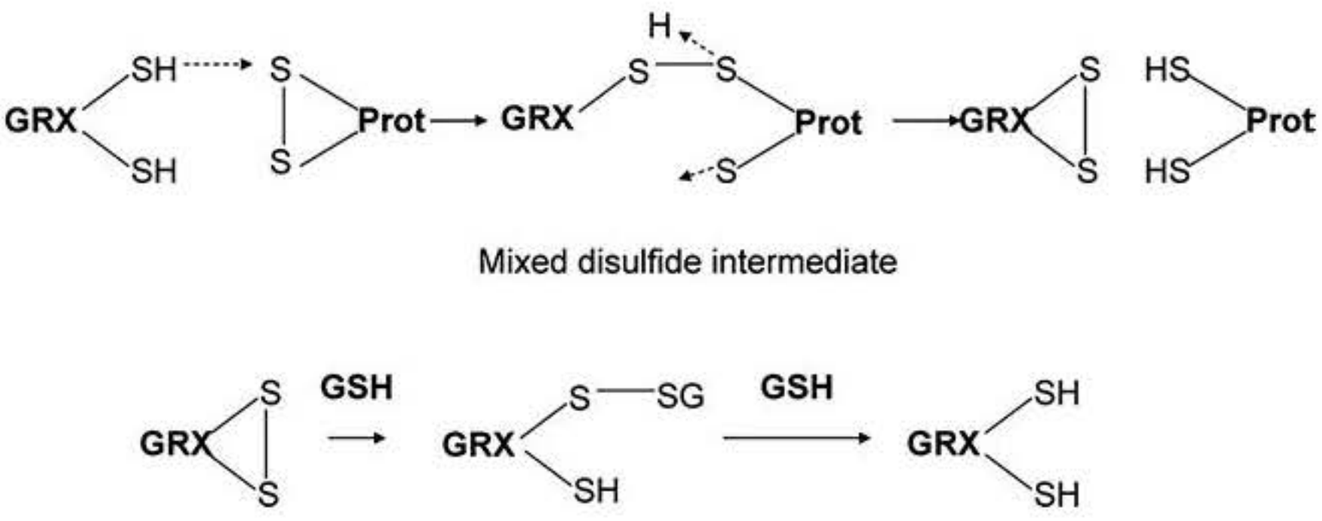

FIG. 5. Reduction of oxidized proteins by glutaredoxin/glutathione (GRX/GSH). Through a mechanism similar to that used by TRX, GRX also forms a mixed-disulfide intermediate with an oxidized protein. This disulfide is resolved through involvement of a second Cys residue of the CXXC motif in GRX, resulting in reduction of the protein and formation of a disulfide bond in GRX. GSH directly reduces GRX again through a disulfide-exchange mechanism and is itself reduced by glutathione reductase. [Adapted from Lillig *et al.* (121).]

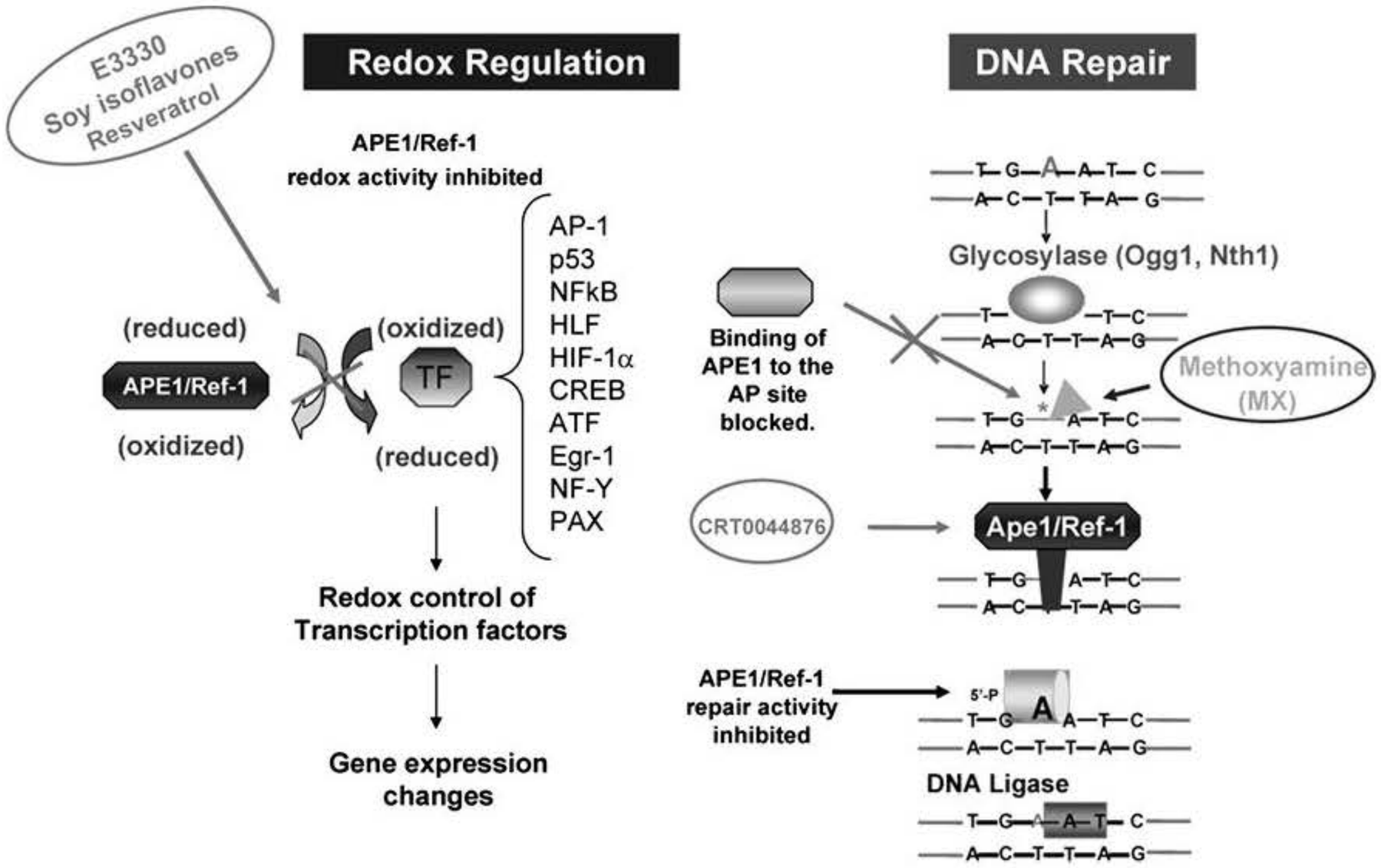

FIG. 6. APE1 has dual roles in redox and DNA repair. APE1 possesses two major functions: redox regulatory/signaling and DNA repair. Through its redox function, APE1 regulates gene expression by modifying the redox status of some transcription factors involved in variety of cancer processes. Small molecules that block APE1 redox function are shown in *ovals*. In addition to its redox function, APE1 plays a critical role in the BER DNA-repair pathway as an AP endonuclease, which processes the AP sites. Blocking AP sites by using methoxyamine (MX) or APE1 or both directly by using APE1-specific inhibitors such as CRT0044876 may decrease DNA repair and lead to tumor-cell death. [Adapted from Luo *et al.* (128).]

but also very similar endonuclease active sites (Fig. 8). The sequence identity between APE1 and exonuclease III is 27.7%. The most obvious structural difference between human APE1 and exonuclease III is an additional 62 N-terminal residues found only in APE1 (Fig. 7). Within this N-terminal region of APE1 is a nuclear localization sequence. However, addition of N-terminal residues alone does not confer redox activity; zebrafish APE includes a similar N-terminal addition (Fig. 9) but lacks redox activity (68).

So the question then becomes, what is required for the redox activity of human APE1 (hAPE1)? This continues to be a source of controversy in the literature. Of the seven Cys residues present in hAPE1, Cys 65 was identified as the critical residue required for redox activity through analysis of single Cys-to-Ala substitutions within APE1 (see Fig. 10) (178). Investigation of the role of Cys residues within APE1 was based on the finding that a Cys residue within the DNA-binding domain of the transcription factor c-Jun was subject to oxidation, leading to loss of DNA bindings and was reduced by APE1 (2, 184, 187). Subsequently, the crystal structure of human APE1 was reported (70), revealing that Cys 65, a residue unique to mammalian sequences, is a buried residue located on the first β strand in the fold, which is part of a β sheet in the core of the protein (Fig. 10). The residue equivalent to the hAPE1 Cys 65 in exonuclease III, based on structural alignment, is Val 4, whereas that in zebrafish APE1 (zApe) is Thr 58 (68). Conservation of Cys residues between

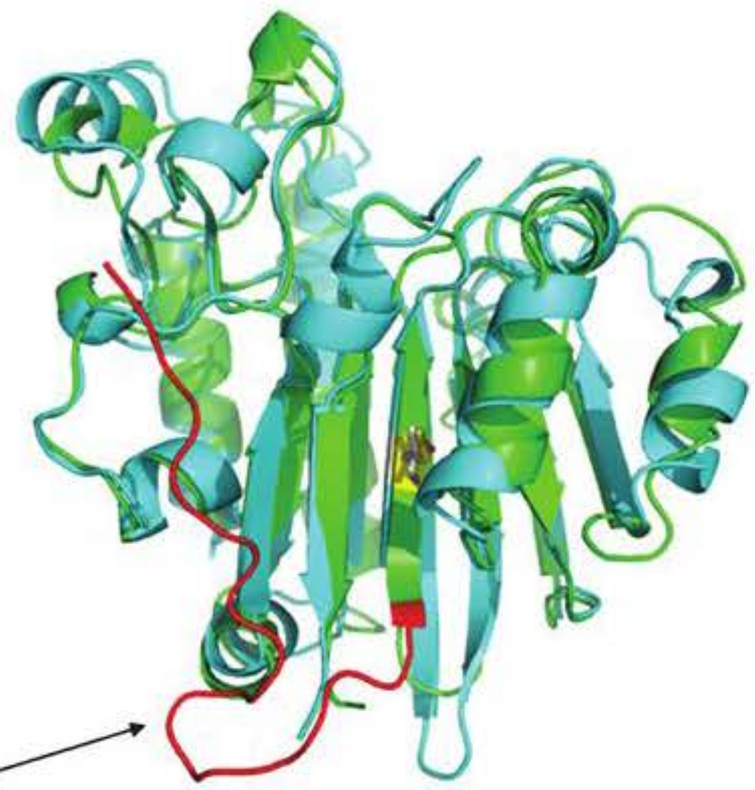

FIG. 7. Comparison of human APE1 and exonuclease III from *Escherichia coli*. The structurally similar enzymes, human APE1 (PDB identifier, 1BIX, green ribbon rendering) and exonuclease III (PDB identifier, 1AKO, blue), share a similar fold; however, APE1 includes an additional 62 N-terminal residues, highlighted in red (residues 44-62). In exonuclease III, the residue equivalent to the APE1 Cys 65 (*yellow stick* rendering) is Val 4 (*gray stick* rendering). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

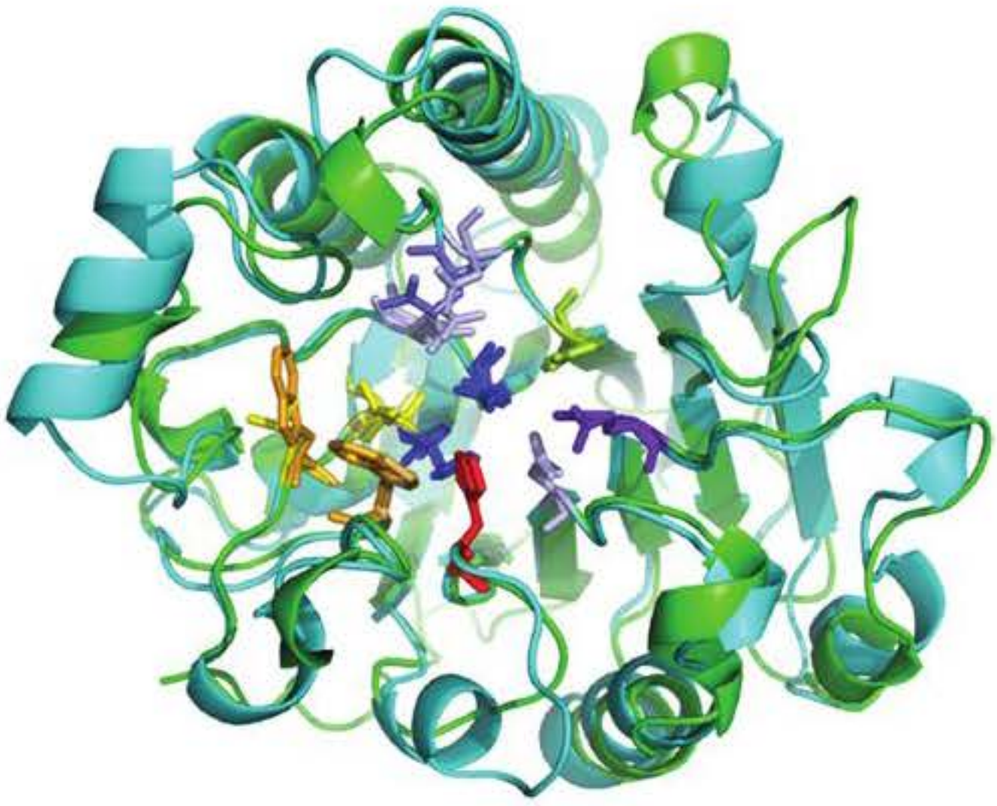

FIG. 8. Comparison of active-site regions of human APE1 and exonuclease III. Stick renderings are shown for residues within the active sites of APE1 (PDB identifier, 1BIX, green ribbon rendering) and exonuclease III (PDB identifier, 1AKO, blue). Active-site residues are color coded as follows: light blue, Asn; slate blue, Gln; blue, Asp; purple blue, Glu; red, His; green, Tyr; light orange, Phe; bright orange, Trp; yellow, Leu; pale yellow, Ile. The active-site regions of APE1 and exonuclease III are highly conserved. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

the hAPE1 and the *E. coli* enzyme is limited to Cys 208 and Cys 310, but within vertebrate APEs, all Cys residues except Cys 65 and Cys138 are conserved (68). The structure of APE in vertebrates also is conserved, based on a comparison of the zAPE and human APE1 structures. Within the vicinity of Thr

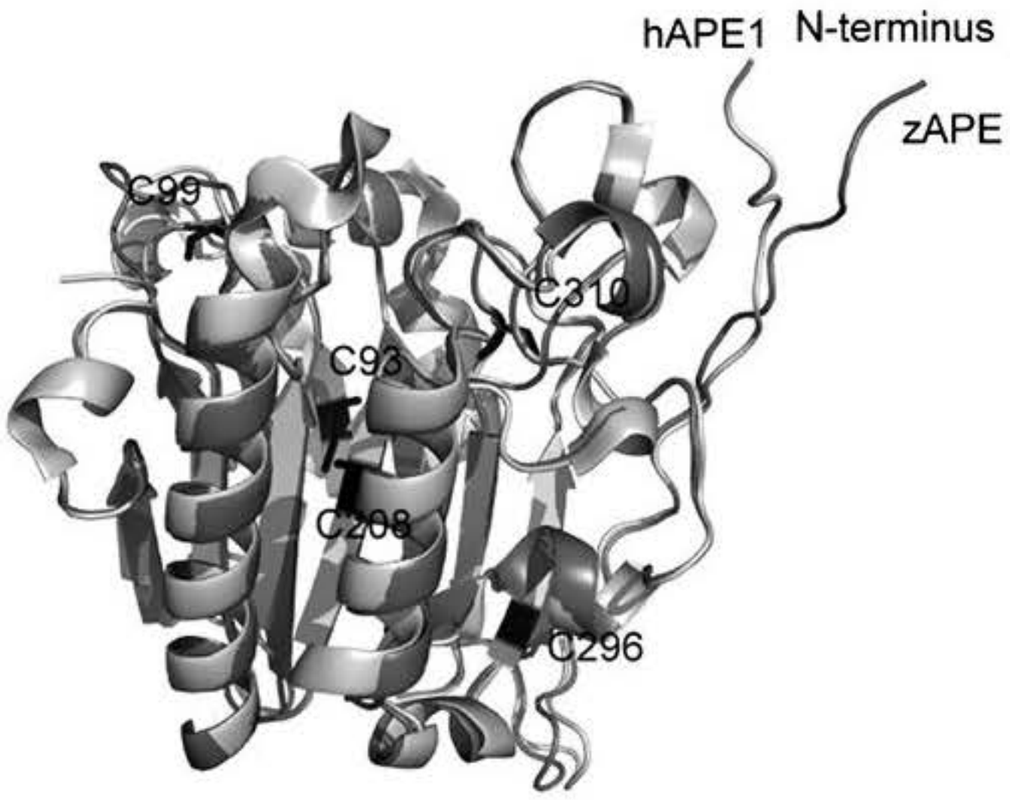

FIG. 9. Comparison of zebrafish APE and human APE1. Although the zebrafish APE (PDB identifier, 203C, *light gray*) also includes an extended N-terminus similar to that found in the human APE1 (PDB identifier, 203H, *dark gray*), it does not have redox activity. As shown in these ribbon renderings, the zebrafish and human enzymes are structurally very similar, including the N-terminal residues. Conserved Cys residues, including 93, 99, 208, 296, and 310, are shown in *black stick* renderings for the human enzyme.

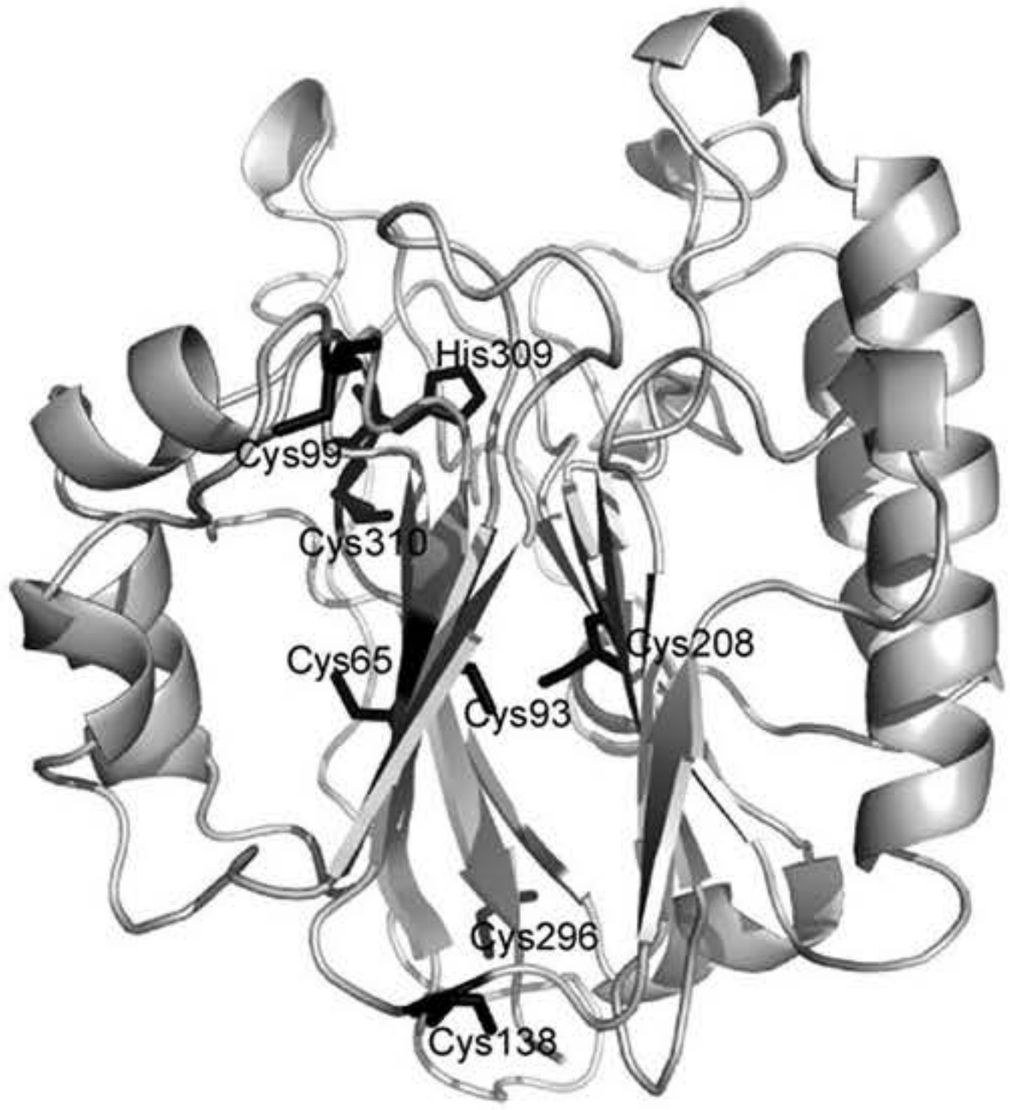

FIG. 10. Positions of Cys residues within APE1. The human APE1 (PDB identifier, 1BIX, *gray ribbon* rendering) includes seven Cys residues (*black sticks*), whose positions relative to the active-site His 309 are shown. None of the Cys residues is appropriately positioned to form a disulfide bond, and the redox-critical Cys residue, Cys 65, is a buried residue located in the first β strand of the APE1 fold.

58, the structure and chemical environment is highly conserved (68). In contrast, residues in the vicinity of Val 4 in the *E. coli* enzyme exonuclease III are not similar to those found in the vertebrate APEs.

The report of a viable C64A knockin mouse and data within challenged the validity of a role for Cys 65 in the redox activity of APE1 (148). However, we note that the mouse knockin study does not directly address the role of Cys 65 (64 in mouse) in the redox activity, but rather suggests that Cys 65 is not essential for the development of the mouse, potentially confirming that redox systems are redundant. In an effort to clarify this issue, we substituted Thr 58 with Cys in zAPE, thereby conferring redox activity to the enzyme, as measured both in EMSA redox and transactivation redox assays (68). To date, the preponderance of evidence supports a role for Cys 65 in the redox activity of hAPE1. Thus, we conclude that evolution of redox activity in hAPE1 is coincident with the appearance of Cys 65 in mammalian sequences.

B. Comparison of APE1 with other redox factors

In contrast to molecules such as TRX and GRX, which maintain the general redox status of the cell, APE1 does not contain two Cys residues within a C-X-X-C motif. Thus, the mechanism by which APE1 reduces transcription factors is likely to differ from that of thioredoxin or glutaredoxins. In the crystal structures reported to date of APE1, no disulfide bonds are present, and the only Cys residue reported to be absolutely required for redox activity is Cys 65, which is a buried residue. The Cys residues positioned closest to one

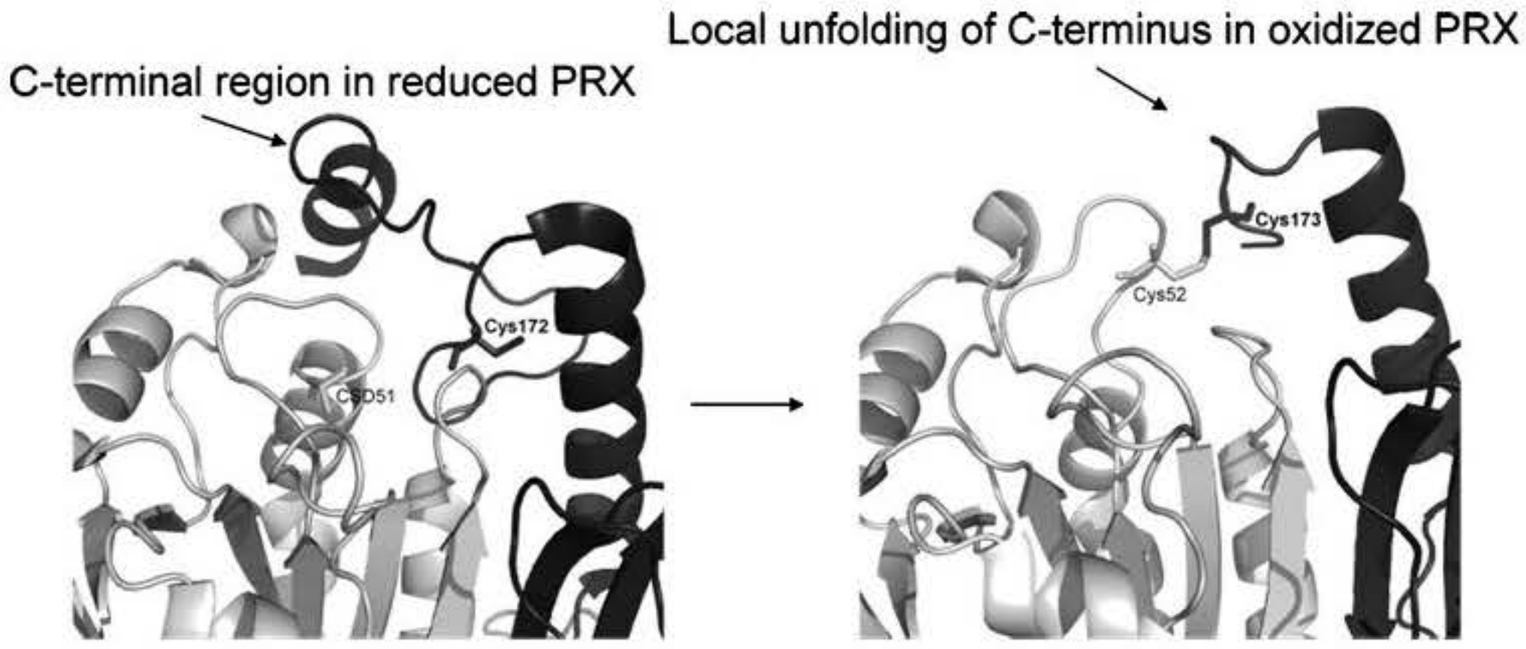

FIG. 11. Redox mechanism of peroxiredoxin (PRX) involves local unfolding. Peroxiredoxin is the enzyme responsible for detoxification of hydrogen peroxide. In a mechanism dissimilar to that used by TRX or GRX, PRX has two Cys residues, one located in each subunit of the dimeric structure, which participate in the reduction of hydrogen peroxide, leading to the formation of disulfide bond in PRX. Reduced PRXII (PDB identifier, 1QMV) is shown in the *left panel* as a ribbon rendering with one subunit in light gray, the second in dark gray, and Cys residues in black. In the *right panel*, the oxidized PRXI (PDB identifier, 1QQ2) is rendered similarly. Unfolding of the C-terminus of the dark-gray subunit allows the formation of a disulfide bond between the Cys residues that are located ~9 Å apart in the reduced form of the protein.

another are 93 and 208, but their respective S atoms are ~3.5 Å apart, too far apart to form a disulfide bond, which is typically ~2.2 Å in length. Further, these residues are buried in the core of the protein and are not accessible. The solvent-accessible Cys residues include 99 and 138; however, these residues are not in close proximity to one another and would not be expected to interact to form a disulfide bond. Furthermore, substitution of Ala for either Cys 99 or 138 has no effect on redox activity (178). Before the determination of the crystal structure of APE1, it was proposed that Cys 65 and 93 form a disulfide bond (178). Given the respective locations of Cys 65 and Cys 93 in the protein, >8 Å apart and positioned on opposite sides of the β sheet (Fig. 10), a substantial conformational change in the structure of the protein would be required for a disulfide bond to form between these residues.

Another group of redox proteins, peroxiredoxins, are responsible for sensing hydrogen peroxide in the cell and serve as catalysts to detoxify this extremely reactive molecule (87). These enzymes are dissimilar to thioredoxin- or glutaredoxin-type molecules in that they lack a C-X-X-C motif, but they do include two Cys residues that are required for activity (183). These Cys residues are located ~9 Å from one another in the fully folded dimeric structure (Fig. 11); one Cys from each monomer forms the active site (183). The nucleophilic thiolate is sequestered before a local unfolding event near the dimer interface. The resolving thiolate is located near the C-terminus of the molecule and, on local unfolding, is placed in close proximity to the nucleophilic thiolate (183) (Fig. 11). Peroxiredoxins reduce hydrogen peroxide and not other proteins (87), but on overoxidation to the sulfenic or sulfinic acid state, are themselves reduced by sulfiredoxin (29, 177). The requirement for local unfolding for peroxiredoxin to complete its catalytic cycle in the detoxification of hydrogen peroxide is very interesting and may be a general mechanism used by other redox factors. Although APE1 does not include two Cys residues positioned similarly to those found in peroxiredoxin, the possibility remains that a conformational change or local unfolding event may result in more favorable positioning of Cys residues. An interesting similarity between APE1 and peroxiredoxin is the proximity of a Cys residue to a terminus; in the case of hAPE1, Cys 65 is located relatively close to the N-terminus of the protein; it is located within the first secondary structural element (a β strand) in the fold (Fig. 7). In summary, we conclude that hAPE1 is unique as a redox factor, having evolved this additional function while maintaining its essential base-excision repair activity.

C. Mechanism of redox regulation by APE1

Although a role for Cys 65 in the redox activity of hAPE1 has been established (68, 178), a detailed mechanism has yet to be elucidated. It is possible that another as-yet-unidentified Cys residue in APE1 is involved in the redox activity. In this case, after the formation of a mixed disulfide intermediate between APE1 and a transcription factor, a resolving Cys would serve to restore the thiolates in the transcription factor. As noted earlier, this interaction would likely involve a different conformation of hAPE1 than has been reported in the crystal structures to date (17, 68, 70, 139). Alternatively, a residue other than Cys might be involved in the redox activity, perhaps a Ser residue, as found in some glutaredoxins. A similar mechanism would be proposed in this case, although involvement of a Ser residue would require a significant reduction in its pK_a. As the stoichiometry of the relevant redox complex has yet to be established, it is possible that more than one APE1 molecule is involved in the reduction of transcription factors. In this case, a Cys or Ser from a second molecule of APE1 may serve as the resolving thiolate, again, in a mechanism similar to that proposed for thioredoxin. As this problem is of considerable interest, we are actively investigating the mechanism of redox regulation by hAPE1.

V. Transcription Factors Regulated by the Redox Activity of APE1

Because APE1 is a multifunctional protein involved in both the repair of DNA damaged by oxidative or alkylating compounds and in the redox regulation of a number of stress-inducible transcription factors, such as AP-1, NF-κB, HIF-1α,

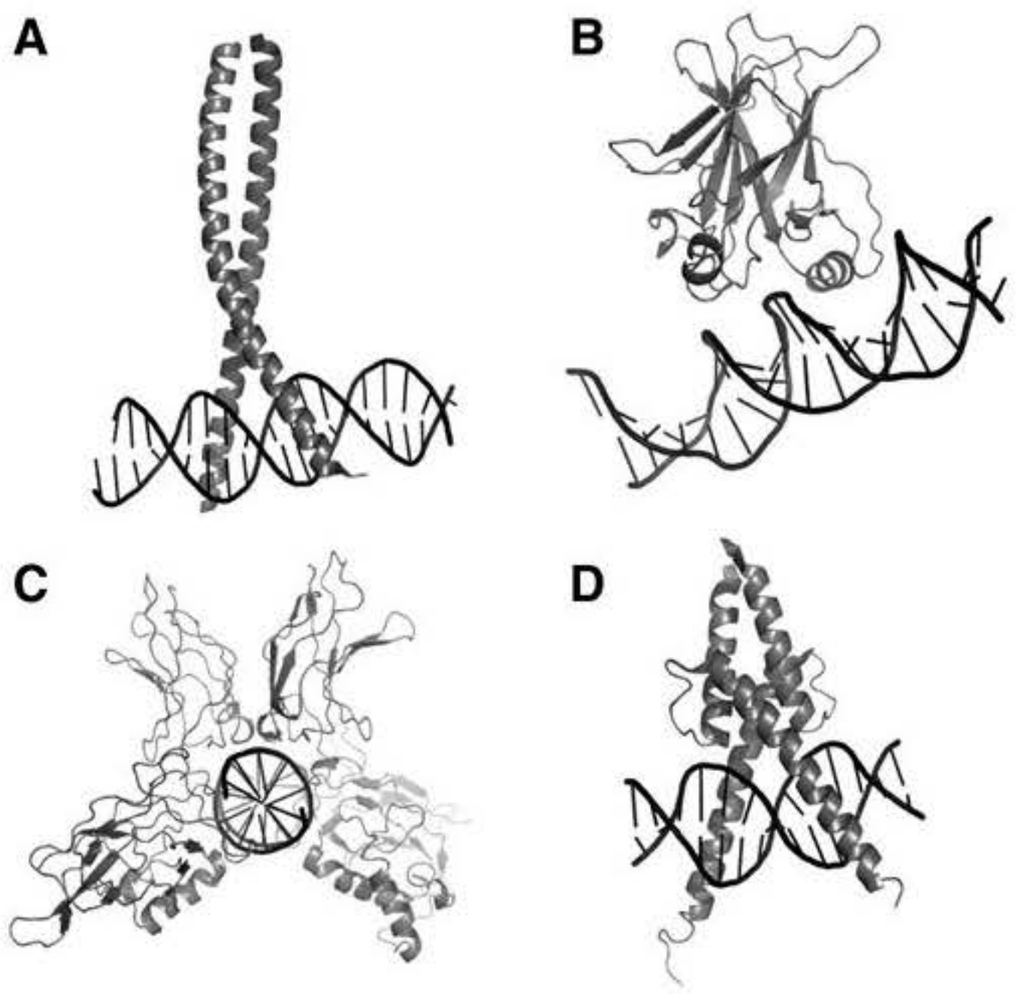

FIG. 12. Structural comparison of DNA-binding domains from transcription factors that are reported to be redox regulated by APE1/Ref-1 bound to DNA. Proteins are shown in a *gray ribbon* rendering, and the DNA in a *spline/stick* representation in black. The DNA-binding domains of the leucine zipper–motif protein AP-1 (c-Jun/c-Fos) bound to DNA (PDB identifier 1FOS) is shown in (**A**), the immunoglobulin-like fold of p53 (PDB identifier 1TSR) with an α helix bound in the major groove (**B**), and the heterodimeric two-domain immunoglobulin-like folds of p65/p50 NF-κB (PDB identifier 1VKX) in (**C**). A representative basic leucine-zipper/basic helix-loop-helix–containing protein (MyoD) bound to DNA (PDB identifier 1MDY) is shown in (**D**) to illustrate the type of interaction expected for HIF-1α bound to DNA.

and p53, it clearly plays a pivotal role between redox signaling that alters the cellular response to DNA damage and DNA-repair functions. These transcription factors are multidomain proteins that are structurally unrelated, suggesting that no common structural motif is recognized by APE1. Further, the DNA-binding domains of these factors, which are redox regulated, are structurally distinct (Fig. 12); AP-1 is a heterodimeric bZip family protein; p53, a single immunoglobulin-like domain; NF-κB, a dimer of two-domain immunoglobulin-like subunits; and HIF-1α, a basic helix-loop-helix domain. Redox regulation of transcription factors serves as one of several mechanisms that control sequence-specific DNA-binding and thereby gene expression. Of the transcription factors regulated by APE1 that play an important role in the DNA-damage response or DNA-repair pathways including DR, BER, HR, MMR and GGR, p53, AP-1, and HIF-1α are discussed here.

A. p53

The tumor-suppressor p53 is a sequence-specific transcription factor that serves as a potent tumor suppressor and functions in part by preserving genomic integrity (113). Not surprisingly, it has been demonstrated that p53 promotes genomic integrity by regulating some of the DNA-repair pathways (80, 162). Regulation of DNA repair by p53 is complex, involving both transactivation-dependent and -independent mechanisms, reviewed by Sengupta and Harris (162), and p53 is itself regulated by both redox-dependent and -independent mechanisms involving Ref-1 (APE1).

Investigation of redox regulation of p53 was initiated based on the finding that oxidized p53 bound DNA very poorly (75) and led to the discovery that Ref-1 was the factor responsible for enhancing the DNA-binding activity of wild-type p53 (101). Although Ref-1 is believed to interact transiently with p53, and this interaction has been detected only by far Western analysis (67) and not by coimmunoprecipitation (101), Ref-1 stimulates DNA-binding activity *in vitro* and transactivation *in vivo* by wild-type p53 (67, 101). The mechanism by which Ref-1 enhances the DNA-binding activity of p53 has not yet been fully elucidated but has been proposed to include redox-dependent activation of the DNA-binding domain, potentially through direct reduction of a disulfide bond, as well as a redox-independent interaction with the C-terminal regulatory domain of p53 (101). Most recently, Ref-1 was shown to interact with the tetramerization domain, promoting formation of tetramers from dimers and thereby enhancing sequence-specific DNA-binding activity of p53 through a redox-independent mechanism (77). Redox signaling involving p53 has also been shown to depend on a general redox factor as well as Ref-1. TRX has been shown to enhance stimulation of p53-dependent expression of p21 by Ref-1 suggesting a link between TRX and Ref-1 in cellular response to oxidative stress (175). A summary of p53 regulation of DR, BER, NER, MMR, HR, and NHEJ repair pathways follows.

BER is initiated by highly specialized DNA glycosylases that cleave the DNA base, creating an AP site, as discussed earlier. Regulation of the BER pathway by p53 involves both activation and negative regulation of BER enzymes under different conditions and in different cell lines. In response to γ-ray treatment, p53 was demonstrated to promote activation of AAG. However, this initial step in BER was also shown to be under negative transcriptional regulation by p53 after exposure to nitric oxide (NO) (202). Recent studies demonstrated that p53 downregulated APE1 expression through binding to the promoter region of APE1 that includes an SP1 site. APE1 mRNA and protein levels decreased in a time-dependent manner in the human colorectal cancer line HCT116 p53(+/+), but not in the isogenic p53-null mutant after treatment with camptothecin. Overexpression of wild-type p53 in the p53-null cells significantly reduced both endogenous APE1 and APE1 promoter–dependent luciferase expression in a dose-dependent fashion (196). Thus, Ref-1 (APE1) regulates DNA-binding of p53, and p53 in turn regulates both expression and protein levels of APE1 in this example.

Another BER enzyme, DNA polymerase β (*pol* β), involved in SP-BER, has been shown to be regulated by p53. *Pol* β has associated dRP lyase activity that is important and often rate limiting in BER and acts after APE1 activity. *Pol* β also plays a role in single nucleotide gap filling in LP-BER. In p53-deficient cells, it has been shown that *pol* β protein expression is altered (35). Additionally, *pol* β gene expression in CHO cells and HeLa cells can be upregulated by CREB in response to DNA alkylating agent exposure (79). As discussed, CREB is under redox control for its binding to DNA and activating transcription. Therefore, a tight link and interaction occurs in the

Table 1. Role of APE1 in Regulating Expression of DNA Repair Genes

Ape1/Ref-1 Transcription Factor				
NFkB	*AP-1*	*CREB*	*p53*	*HIF-1α*
Direct Repair			*Direct Repair*	
AGT			AGT	
	Homologous Recombination		*Homologous Recombination*	*Homologous Recombination*
	ATM		RAD51	NBS1
	RAD50		WRN	
	Global Genome Repair		*Global Genome Repair*	
	ERCC1		XPC	
	XPA		DDB2	
	RAD23B			
	ERCC3			
	Mismatch Repair		*Mismatch Repair*	*Mismatch Repair*
	MLH1		MLH1	MSH2
	MSH2		MSH2	MSH6
	PMS2		PCNA	
	MSH6		PMS2	
	Base Excision Repair	*Base Excision Repair*	*Base Excision Repair*	
	UNG2	*Polβ*	*Polβ*	
			APE1	
			AAG	

DNA BER process between a number of transcription factors that are under redox control and the DNA-repair response (Table 1).

NER, which is divided into TCR and GGR, as discussed earlier, is affected differentially by p53. For example, several studies found that p53 selectively affected GGR, but not TCR. Two main proteins in GGR, DDB2 and XPC, which are involved in DNA-damage recognition, are transcriptionally regulated by p53 (3, 96, 168). Loss of p53 and subsequent deficiencies in the GGR proteins DDB2 and XPC appear to lead to genome instability. This has been effectively demonstrated in knockout mouse studies. In this study, 100% of $XPC^{-/-}$ mice develop lung cancer, and $DDB2^{-/-}$ mice develop skin tumors (89). Again, if p53 is not fully functional through redox modification, it cannot turn on DDB2 and XPC, which would result in defective GGR (Table 1).

MMR, which is in charge of DNA repair after DNA polymerase errors, removes mismatches in DNA. MSH2 in complex with MSH6 or MSH3 is active in the recognition of single-base mismatches and short insertion/deletion mispairs or larger loops of unpaired nucleotides, respectively. MSH2, MLH1, and PMS2 have all been shown to be under p53 regulation, similar to DDB2 and XPC in NER (38, 159). PCNA, another member of the NER pathway, also is under p53 regulation (Table 1) (189).

DSBs threaten severely genomic stability by facilitating deletion or translocation or both of chromosomal DNA. Because either a deficit or an excess in HR may lead to genomic instability, it is not surprising that HR is highly regulated. Once again, p53 plays an important role in the repair of DSBs through the regulation of both DSB repair pathways, HR and NHEJ. Increased levels of HR have been observed in mice that are deficient in wild-type p53 (26, 127). Arias-Lopez *et al.* (10) demonstrated that p53 inhibits HR through repression of RAD51 expression. Additionally, p53 has been demonstrated to repress the transcription of the RecQ4 helicases, WRN and RecQ4 (163, 191).

Finally, some studies in murine fibroblast cell lines have demonstrated that AGT also is under p53 regulation (72, 155). AGT also appears to be regulated by NF-κB, another transcription factor that is under redox control by APE1. This was demonstrated by overexpression of the p65 subunit of NF-κB in HEK293 cells, resulting in an increase in AGT expression (116).

All of these studies point to the possible relevance of redox controlling DNA-repair responses through p53. Therefore, if reduced p53 is required to bind DNA and either activate or repress the transcription of DNA-repair genes, as discussed earlier, then it follows that Ref-1 (APE1) plays an important role not only in the redox modulation of p53 but also in the regulation of DNA repair.

B. AP-1

Activator protein-1 (AP-1) refers to a family of structurally and functionally related basic leucine zipper proteins (bZIPs) that intermix to form heterodimeric sequence-specific DNA-binding proteins, including primarily Jun proteins, c-Jun, JunB, and JunD, Fos proteins, c-Fos, FosB, Fra-1 and Fra-2, and some ATF-family members, ATFa, ATF-2, and ATF-3 (82). These proteins recognize AP-1 sites that are also referred to as tetradecanoylphorbol-13-acetate (TPA)-responsive elements. AP-1 transcription factors are inducible factors that respond to environmental changes, including stress and radiation, or to growth-factors signals. Proliferation, differentiation, apoptosis, and transformation are some of the processes that are mediated by AP-1 (82).

The first report of redox activity associated with APE1 and its identification as Ref-1 resulted from efforts to identify the nuclear factor responsible for reducing AP-1 (c-Jun/c-Fos) and thereby enhancing its DNA-binding activity (1, 184). Redox regulation of AP-1 was found to result from oxidation/reduction of conserved Cys residues within the basic DNA-binding domains of c-Jun and c-Fos (AP-1) (2). Oxidized AP-1

shows little affinity for DNA as compared with reduced AP-1. c-Fos and c-Jun dimerize through a coiled-coil interaction involving the leucine-zipper motifs and bind DNA as a heterodimer; c-Jun also forms a homodimeric species with lower DNA-binding affinity than the heterodimeric c-Jun/c-Fos, whereas c-Fos does not exhibit any DNA-binding activity on its own (2). The conserved Cys residue within c-Jun is mutated to a Ser residue in the oncogenic v-Jun, which is constitutively active and exhibits enhanced DNA-binding relative to c-Jun (2). Thus, Ref-1 regulates DNA binding of AP-1 in a redox-dependent manner involving direct reduction of the conserved Cys residues within the DNA-binding domains of c-Jun and c-Fos. Ref-1 has been shown to copurify with AP-1 (184) and thus interacts more stably with this transcription factor than with p53. TRX has also been identified as a factor modulating transcriptional activation of AP-1 through its reduction of Ref-1 (83). As summarized later, AP-1, which is rapidly induced in response to a number of cellular stimuli, regulates the expression of several proteins involved primarily in NER and MMR DNA-repair pathways.

In one example of regulation of DNA-repair proteins by AP-1 composed of c-Jun and ATF2, Hayakawa *et al.* (78) identified 23 DNA-repair or repair-associated genes whose promoters are bound on phosphorylation of ATF2 and c-Jun after cisplatin treatment. These genes were identified by using chromatin immunoprecipitation (ChIP) with antibodies against ATF2 and c-Jun, followed by hybridization to promoter arrays (78). These include ERCC1, ERCC3, XPA, MSH2, MSH6, RAD50, RAD23B, MLH1, PMS2, UNG2, and ATM. Confirmatory studies directly established expression of some genes, including ERCC1, ERCC3, XPA, RAD23B, and MSH2, and some genes that have been specifically implicated in the repair of DNA-cisplatin adducts, such as RAD23B, XPA, ERCC3, XPF-ERCC1, MSH2, and PMS2. DNA adducts induced by cisplatin are repaired mainly by the NER pathway. Several important proteins including XPA, RAD23B, ERCC1, and ERCC3 in NER were observed on the promoter array. The members of the MMR complex, including MSH2, MSH6, MLH1, and PMS2, which are included in a large complex involved in the recognition of DNA-cisplatin adducts, are bound strongly by activated c-Jun or ATF2 or both. AP-1 also was discovered to upregulate MSH2 expression in the myeloid leukemia U937 cell line treated with a phorbol ester (TPA) (95). Undeniably, ERCC1 has been recognized to be regulated by AP-1. Li *et al.* (119) demonstrated that AP-1 is transcriptionally up-regulated ERCC-1 in response to TPA in human ovarian cancer cells (119).

All of these studies support the role of the involvement of AP-1 in regulating a significant number of DNA-repair proteins that are involved mainly in the NER and MMR pathways (Table 1). Because AP-1 must be converted from an oxidized to a reduced state to bind to its target sequence, redox control of this protein would have significant implications. As for p53, APE1 is implicated as a potential point of control for regulating the DNA-binding activity of AP-1 and thereby modulating the expression of DNA-repair proteins.

C. HIF-1α and hypoxia

Hypoxia-inducible factor −1 (HIF-1) is a heterodimeric transcription factor comprising HIF-1α and HIF-1β (also known as aryl hydrocarbon–receptor nuclear translocator) subunits (93) that plays an important role under hypoxic conditions in the cell. Of the two subunits, HIF-1α has been shown to be crucial for regulating cellular response to hypoxia and is frequently overexpressed in human cancers. Under normal oxygen conditions, HIF-1α is targeted for ubiquitin-mediated proteasomal degradation by von Hippel-Landau protein (pVHL) (97, 98, 132). Under hypoxic conditions, HIF-1α translocates to the nucleus and dimerizes with HIF-1β, forming HIF-1. HIF-1, along with coactivators, binds hypoxia-response elements (HREs) within promoters and regulates the expression of its downstream genes, including vascular endothelial growth factor (VEGF) (62, 102). The DNA-binding activity of HIF-1 has been shown to be regulated by redox signaling, and the redox-dependent stabilization of the HIF-1α protein is required for activation of HIF-1 (93). Overexpression of TRX and Ref-1 enhanced the DNA-binding activity of HIF-1, as detected in a reporter assay. The results, taken together, suggest a role for Ref-1 as a redox regulator of HIF-1α. Ref-1 (APE-1) also has been shown to be required for the binding of transcriptional proteins to the HIF-1 DNA-recognition element within the rat pulmonary artery endothelial cell VEGF gene (198). Although this study did not specifically address the role of redox regulation, the authors found that Ref-1 was required for the formation of the hypoxia-inducible transcriptional complex, including HIF-1 and transcriptional coactivators, p300 and cyclic AMP response element–binding protein (CREB). In a second example of Ref-1 as a coactivator, a transcriptional complex including HIF-1α, signal and transducer of transcription 3 (STAT3), CREB-binding protein (CBP)/p300, and Ref-1 (APE1) was reported to regulate the protein tyrosine kinase Src-dependent expression of VEGF in response to hypoxia in pancreatic and prostate carcinomas (71). More recently, HIF-1α was shown to play a role in downregulating mRNA and protein levels of Ref-1 under hypoxic conditions in human microvascular endothelial cells (125). Thus, HIF-1α regulates expression levels of Ref-1 and is itself regulated by Ref-1.

Increasing evidence reveals that hypoxic stress in the tumor microenvironment can cause genetic instability in cancer cells. Hypoxia induces changes in the expression of several genes involved in DNA-repair pathways. These studies suggest that hypoxia downregulates the expression of key genes within the MMR pathway, including MLH1 and MSH2, and several critical mediators of HR, BRCA1, BRCA2, and RAD51, resulting in significant genetic instability (21, 22, 24, 25, 32, 109, 135, 138) [reviewed in Bindra *et al.*, 2007(23), and Bristow and Hill, 2008 (32)]. The MMR genes appear to be repressed through a mechanism involving c-Myc (25).

In most studies, repression of MMR and HR has been shown to be independent of HIF-1α (21, 22, 24, 25, 32, 135, 138). However, this has been contradicted by Koshiji *et al.* (109), who demonstrated that HIF-1α is responsible for genetic instability during hypoxia at the nucleotide level by inhibiting MSH2 and MSH6, which recognize DNA base mismatches. These investigators demonstrated that HIF-1α displaces the transcriptional activator Myc from Sp1 binding to repress MSH2-MSH6 in a p53-dependent manner (109). HIF-1α also has been shown to be associated with the loss of MSH2 expression in human sporadic colon cancers (172), linking hypoxia to DNA repair in the induction of these cancers. The decrease of the expression of NBS1, a member of the MRe11–RAD50–NBS1 (MRN) complex that initially recognizes DNA

DSBs, has also been shown to be HIF-1α dependent (173). These studies demonstrate that the regulation of DNA repair is an integral part of the hypoxic response and that hypoxia affects DNA repair partially through HIF-1α, a critical mediator in the hypoxic response (Table 1). As Ref-1 has been implicated in the redox regulation of HIF-1α, it then also plays a role in the regulation of DNA-repair genes controlled by HIF-1α.

VI. The Multifunctional APE1 and Redox Control

APE1 is a vital multifunctional protein that acts as an essential master regulator contributing to the maintenance of the genome stability (Fig. 6). Functional activities associated with APE1 include apurinic/apyrimidinic endonuclease activity essential for BER, redox activity, transcriptional regulatory activity (19), and most recently, RNA- cleavage activity (15, 176). In this review, we have limited our discussion to the APE1 BER repair and redox activities, the functions relevant to redox regulation of DNA repair. APE1 also is subject to a number of interesting posttranslational modifications, including acetylation, phosphorylation, and nitrosylation. The implications of these modifications were reviewed recently (19, 171) and are not further discussed here.

APE1 has a pleiotropic role in controlling cellular response to oxidative stress. In addition to its repair function in BER as an AP endonuclease, APE1 controls the redox status of either ubiquitous (*i.e.*, AP-1, Egr-1, NF-κB, p53, CREB, HIF-1α) or tissue-specific transcription factors (*i.e.*, PEBP-2, Pax-5 and −8, and TTF-1) (Fig. 6) (6, 35, 48, 84, 94, 112, 169, 175, 184, 185).

ROS induced by different oxidative or toxic agents have been shown to increase APE1 expression transiently (Fig. 13). A number of transcription factors, including Egr-1, CREB, and Jun/ATF4, are involved in the inducible expression of APE1 (66, 73, 152). In a recent study, the level of APE1 was shown to be increased transcriptionally in response to ROS in melanoma cells by microphthalmia-associated transcription factor (MiTF), a key transcription factor for melanocyte lineage survival that plays an important role in development and carcinogenesis (122). In this study, melanoma cells were classified into MiTF-positive and -negative groups to explore the function of MiTF in regulating cellular responses to ROS. A high level of APE1/Ref-1 was discovered in the MiTF-positive melanoma cell lines. Knocking down MiTF reduced the APE1 protein level and abolished induction of APE1 by ROS. MiTF-negative melanoma cells survived more poorly under ROS stress than did the MiTF-positive cells. Overexpression of APE1 partially rescued ROS-induced cell death when MiTF was depleted. The MiTF regulation of APE1 is direct through E-boxes on the APE1 promoter. It also was found that exposure of HeLa cells to H_2O_2 and to histone deacetylase inhibitors increases acetylation of APE1 at residues Lys6/Lys7, leading to Egr-1–mediated induction of the tumor-suppressor PTEN gene expression (52). In addition, increasing evidence demonstrates that functional triggering of membrane-bound receptors (such as TSH, CD40L, ATP,

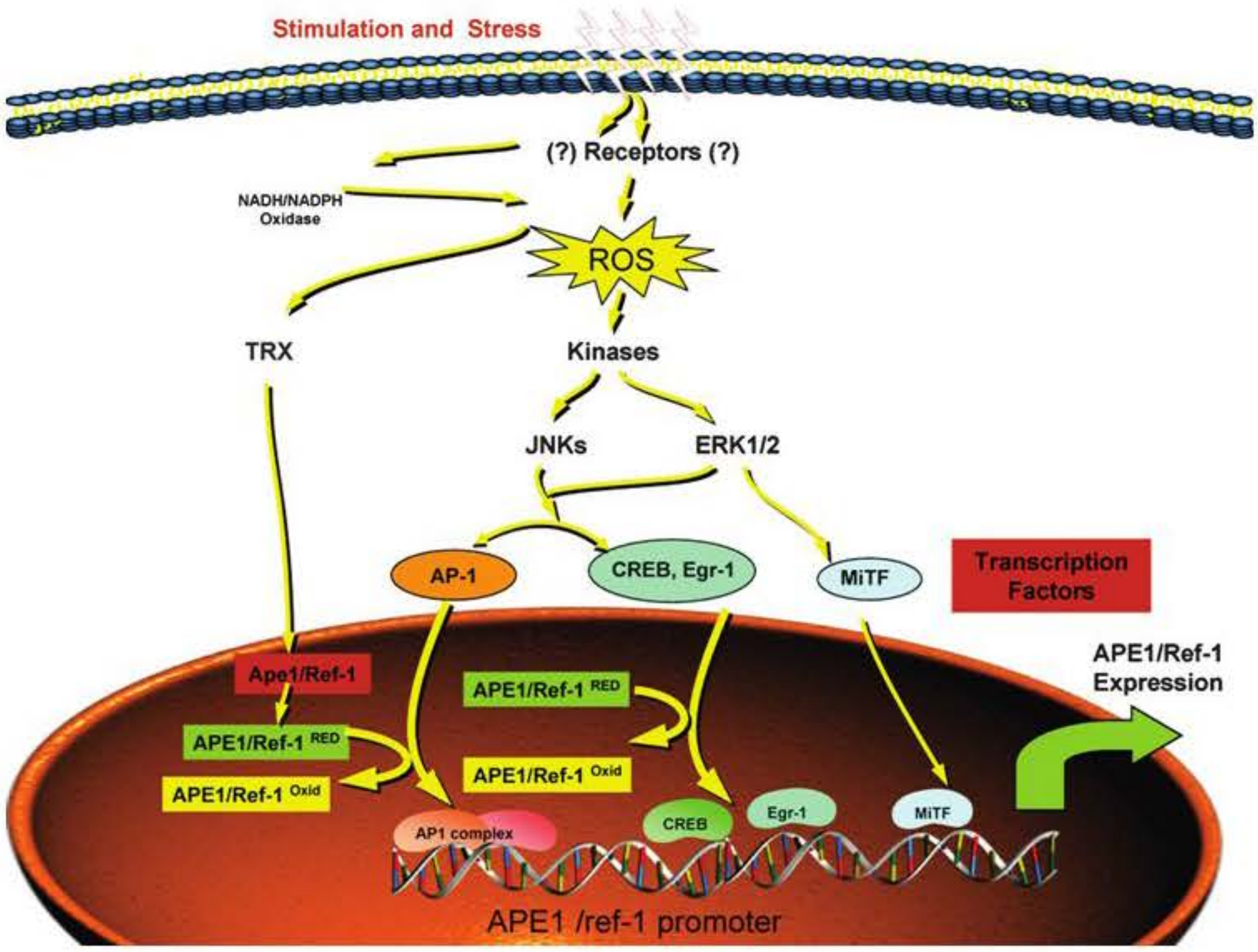

FIG. 13. Overview of ROS signaling. Reactive oxygen species (ROS) are generated through external stimuli or stress, as well as metabolic processes, and act as a signal for JNK and ERK1/2 kinases, resulting in the activation of a number of transcription factors, including Egr-1, CREB, and Jun/ATF4, which are involved in the inducible expression of APE1. In melanoma cells, MiTF also was shown to upregulate transcription of APE1. In its role as a redox factor, APE1 reduces a number of transcription factors, including AP-1, Egr-1, NF-κB, p53, CREB, and HIF-1α. Thus, APE1 controls the redox status of several transcription factors that in turn regulate expression of APE1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

IL-2) can lead to APE1 functional activation through intracellular generation of sublethal doses of ROS (170). APE1 also is directly responsible for the control of the intracellular ROS levels through inhibiting the ubiquitous small GTPase Rac1, the regulatory subunit of NADPH oxidase system (9, 149). Recently, Park *et al.* (151) found that overexpressing APE1/Ref-1 increased inhibition of angiotensin II (Ang II) to the whole-cell conductance Ca^{2+}-activated K^+ (BKca) currents in human umbilical vein endothelial cells (HUVECs) through blocking NADPH oxidase–dependent ROS production. The inhibitory effect of Ang II on BKca channel function is NADPH oxidase dependent (151). It also was demonstrated that NADPH-mediated ROS production induced by the P2Y purinergic receptor triggering was able to promote APE1 functional activation (152) and results in the proposition of an autoregulatory loop between these two systems.

We demonstrated that reducing expression of APE1 in neuronal cultures by using small interfering RNA (siRNA) enhances cisplatin-induced ROS generation, cell killing, and apoptosis (103, 136). Another recent study showed that Ape1 can antagonize the generation of ROS. Overexpression of APE1 inhibits, whereas silencing APE1 expression potentiates ROS accumulation under treatment with oxidative reagents or loading with granzyme K in cytotoxic T lymphocyte (CTL)/natural killer (NK) cells. APE1 is a physiological substrate of granzyme K, and cleavage by granzyme K facilitates intracellular ROS accumulation and enhances granzyme K-induced cell death (74). Merluzzi *et al.* (136) also found that repression of APE1 by antisense overexpression determines an additional increase in CD40-mediated B-cell proliferation, and the increase is abolished by pretreatment of cells with the antioxidant *N*-acetyl-L-cysteine (NAC). They proposed that APE1, through control of the intracellular redox state, may also affect the cell cycle by inducing nucleus-cytoplasm redistribution of p21 (136).

A genetic study has demonstrated that APE1 is essential for cell survival and organism development. Knockout of APE1 in mice leads to postimplantation embryonic lethality on days E5 to E9 (188). Conditional knockout and knockdown strategies also confirmed the crucial role of this protein in cells (58, 65, 99). In addition, studies demonstrated that altering APE1 levels leads to a change of cell growth, survival, and sensitivity (28, 37, 56, 58, 81, 115, 134, 147, 179, 180). However, these studies used either overexpression of APE1, APE1 antisense oligonucleotides, or APE1 siRNA to change the total amount of cellular APE1 and thereby all functions of APE1, including its repair and redox activities. Thus, the function of APE1 involved in each case of altered cellular function cannot be identified. Because APE1 plays complex and critical roles in cell survival, proliferation, differentiation, and apoptosis in physiologic and pathologic conditions, as well as in the growth and development of the organism, it is important to distinguish and characterize which function of APE1 is involved in different biologic events, especially those that may differ in normal cells and various pathologic cells, such as cancer cells. The use of specific small-molecule inhibitors blocking either repair or redox, but not both functions of APE1, will give a clearer picture and will be helpful for modifying its function in treatment of the different diseases. Our recent data demonstrated that E3330, 2E-3-[5-(2, 3 dimethoxy-6-methyl-1,4-benzoquinolyl)]-2-nonyl-2-propenoic acid]), a novel quinone derivative, specifically blocks the redox function of APE1 and has no effect on APE1-repair activity and other members of the BER pathway (128). By using E3330, we demonstrated that APE1 is required in normal embryonic hematopoiesis and that the redox function, but not repair activity, of APE1 is critical in normal embryonic hematopoietic development (199).

VII. Modulating APE1 Activities as a Cancer Therapeutic Approach

Elevated APE1 levels have been demonstrated in a variety of cancers and are typically associated with aggressive proliferation, increased resistance to therapeutic agents, and poor prognosis (50, 140, 180, 190). Previous studies demonstrated that decreasing APE1/Ref-1 levels leads to the blockage of cell growth and the increase of cellular sensitivity to DNA-damage agents by using anti-sense oligonucleotides and siRNA of APE1/Ref-1 (56, 58, 115, 147, 180). Not yet known is the relative importance of APE1 redox *versus* the repair function in cancer. Efforts to determine the effects of inhibiting either the redox or repair function of APE1 are ongoing. Targeting of a specific protein, particularly one that plays an important role in cellular response to stress, by chemical knockout (*i.e.*, through use of a small-molecule inhibitor) may have unintended consequences. However, exploration of novel targets is clearly an avenue that merits pursuit, particularly in the case of cancers for which current treatments are ineffective. There has been considerable debate in the literature regarding the wisdom of inhibiting essential DNA-repair enzymes (14, 42, 57, 106, 129). However, small-molecule inhibitors have been identified for several DNA-repair enzymes, including MGMT, poly ADP-ribose polymerase (PARP1), ataxia–telangiectasia mutated kinase (ATM kinase), APE1, and DNA PKcs (42, 57, 129). Targeting of the redox function of APE1 represents a novel approach in the development of a cancer therapeutic agent. Blocking of the redox function of APE1 would be expected to affect the activity of a number of downstream transcription factors and the gene products that they regulate. In this section, we focus primarily on redox inhibitors, those known specifically to inhibit APE1 and those that may affect APE1 either directly or indirectly. We briefly discuss APE1-repair inhibitors as they have been reviewed recently (106).

A. APE1 redox inhibitors

1. E3330. One molecule previously discussed was demonstrated to bind specifically to APE1 with a binding constant estimated by surface plasmon resonance analysis (SPR) of 1.6 n*M*, which suggests a specific interaction between APE1 and E3330 (166). We recently demonstrated that E3330 blocks the redox function of APE1 with AP-1 as the downstream target *in vitro,* as well as after the treatment of ovarian cancer cells with E3330 (128). Additionally, we found that E3330 blocks APE1 redox activity with HIF-1α and other downstream transcription factors (Table 1). This demonstrates that the redox inhibition is not specific for the downstream target. Although E3330 blocked the redox function of APE1, it had no effect on APE1-repair endonuclease activity. We also found that E3330 does have single-agent cancer cell–killing abilities in a variety of cancer cell lines representing ovarian, colon, lung, breast, brain, pancreatic, prostate, and

multiple myeloma cancers, but does not have significant cell killing in our studies with normal cells, such as embryonic hematopoiesis cells, retinal vascular endothelial cells (RVECs), and human $CD34^+$ progenitor cells. These data implicate the redox role of APE in cancer, but not in "normal" cell survival. Inhibition of the APE1 redox function significantly attenuates RVEC proliferation and capillary formation *in vitro* but does not cause cell death. Furthermore, the capillary formation of RVECs appears much more sensitive to redox inhibition of APE1 than to the proliferation. This is the first time that this role of APE1 has been clearly demonstrated. Additionally, our data demonstrate a new role of APE1 in angiogenesis, and inhibition of APE1 redox function by E3330 abrogates this role (128). E3330 also has been shown to inhibit the growth of pancreatic cancer cell lines, an effect that is enhanced under hypoxic conditions (200), as well as pancreatic cancer–associated endothelial and endothelial progenitor cells (201). Consistent with redox regulation by Ref-1, the DNA-binding activity of HIF-1 is inhibited by E3330 in the aforementioned pancreatic cancer studies. Thus, collectively our data and those of others suggest that APE1 redox function will be a promising target of cancer treatment and will open a new avenue for cancer treatment.

2. Other redox inhibitors. A number of natural products reported to affect either directly or indirectly the redox function of APE1 in cells were recently reviewed (57, 107, 128, 133) and are therefore discussed only briefly here. Soy isoflavones, a component in soybeans, are thought to have potential as chemopreventive agents in prostate cancer (137). Treatment of PC-3 prostate cells and xenograft mice with soy isoflavones after radiation treatment resulted in increased cell killing, reduced NF-κB binding to DNA, and reduced APE1 levels. The authors concluded that the soy isoflavones reduced APE1 levels and subsequently resulted in a reduction of the ability of APE1 to reduce NF-κB, resulting in the inability of the cells to respond to the stress (156). However, at this point, the data are merely correlative.

Another natural product, resveratrol, a component of red wine and grapes, was reported to affect the redox activity of APE1 (193). Resveratrol was shown to inhibit both the endonuclease activity of APE1 and the DNA-binding activities of AP-1 in cellular extracts, presumably through inhibition of APE1 redox function. However, this has not been corroborated by others, nor has it been shown to be effective at levels that are physiologically relevant.

B. APE1 repair inhibitors

To date, two different classes of small molecules have been reported to inhibit the AP endonuclease activity of APE1, methoxyamine (18, 124) and negatively charged molecules including CRT0044876 (130), an aryl stibonic acid 13755 (161), and a number of pharmacophore-based compounds (197). Methoxyamine (MX) blocks repair by reacting with apurinic/apyrimidinic sites in the DNA and forming stable adducts that prevent endonucleolytic cleavage by APE1 (18, 64, 124). As MX acts at the level of the DNA, it would also be expected to block the activity of other DNA-repair enzymes such as DNA polymerase β activity. However, none of the aforementioned compounds has been reported to inhibit the redox activity of APE1; this is not discussed further here. A number of recent reviews discuss the status of these types of agents (14, 57, 106).

VIII. Chemoprevention, Redox Modulation, and DNA Repair

As was clearly documented, DNA damage leading to genome instability is a key step for the initiation and progression of cancer (16). Both endogenous and exogenous DNA-damaging agents and particularly those that induce oxidative stress are some of the main factors that cause DNA damage in cells. Therefore, agents that reduce the oxidative stress and subsequent DNA damage, as well as those that increase the repair of DNA damage, are considered to be pertinent in cancer prevention. It is estimated that nearly one third of all cancer deaths in the United States could be prevented. Accumulating research evidence has shown that some dietary antioxidants are able to reduce the incidence of cancer by increasing DNA repair and reducing oxidative stress.

A. Dietary antioxidants

1. Ellagic acid. Ellagic acid, a component in berries (blueberry, strawberry, and red raspberry), was reported to reduce oxidative DNA damage both *in vitro* and *in vivo* (4, 5). *In vitro*, ellagic acid has demonstrated a >95% inhibition of 8-oxodeoxyguosine (8-oxodG) production. It also was shown to reduce other oxidative DNA adducts caused by 4-hydroxy-17β-estradiol and $CuCl_2$. In an *in vivo* study, female CD-1 mice were fed pure ellagic acid, and formation of DNA adducts was related to ellagic acid in a dose-dependent manner. Further study found both ellagic acid and its natural source resulted in overexpression of genes involved in DNA-repair pathways such as XPA, ERCC5, and DNA ligase III, mainly those involved in NER. These results demonstrated that ellagic acid is effective in preventing oxidative DNA damage both *in vitro* and *in vivo* by increasing DNA repair.

2. Selenium. Selenium is found in plentiful amounts in dairy, eggs, fish, meat, grains, and Brazil nuts. Selenium, in the form of selenocysteine, is a major constituent of many antioxidant enzymes known as selenoproteins. Not surprisingly, Se was reported to be preventive for cancer initiation from oxidative DNA damage through reducing oxidative stress and increasing DNA repair. The active species of Se include hydrogen selenide (H_2Se) and its methylated metabolite, methylselenol (MeSeH), selenomethionine (SeMet), and selenoproteins. Se, in the form of selenomethionine, was reported to promote BER activity by p53 activation in normal human fibroblasts *in vitro* (164). This study demonstrated that Se-induced p53 activation promotes BER activity by reducing specific cysteine residues in p53. A dominant-negative APE1 redox mutant blocks reductive activation of p53 by Se. Se also was shown to stimulate the activity of a selenoprotein, thioredoxin reductase (TR) (165). These data suggest that Se reduces p53 through interactions involving TR, which reduces TRX and APE1, as well as redox interactions between APE1 and p53. Se-induced activation of p53 is also dependent on the BRCA1 protein in recombinational repair, which is frequently mutated in familial breast cancer (55). It also was reported that Se inhibited DNA-binding activity of transcription factors such as AP-1, NF-κB, SP-1, and SP-3, as well

as the DNA-repair proteins XPA in the NER pathway and formamidopyrimidine DNA glycosylase (FPG) in the BER pathway (27, 76, 131, 195). These data imply that Se may reduce cancer incidence through modulating DNA repair, redox status of cells, and the cellular transcriptional response to oxidative stress. It also suggests that the redox function of APE1 is a major component of this interaction. Additional clinical studies have found an inverse relation between the levels of Se and the prevalence of several types of cancer.

3. Oltipraz. Oltipraz (4-methyl-5-(pyrazinyl)-3H-1,2-dithiole-3-thione), a synthetic dithiolethione, is similar to the thiolethione, an antioxidant component in cruciferous vegetables. Oltipraz has been shown to inhibit the development and progression of multiple organ tumors, including breast, bladder, colon, stomach, liver, lymph nodes, lung, pancreas, and skin, induced by a variety of structurally diverse carcinogens in preclinical studies (41). Clinical studies demonstrated that oltipraz has minimal toxicity in humans and significant chemopreventive activity (41). Oltipraz increased NER activity by decreasing platinum-DNA adducts, but not BER activity, as measured by determining the levels of AP sites in HT29 colon adenocarcinoma cells (146). Further study found that oltipraz also increases APE1 protein level and AP-1 DNA-binding, which is partially dependent on APE1 in HT29 cells (194). Oltipraz also was shown to inhibit microvessel formation in both human and rodent bioassays in a dose-dependent manner (158). These data suggest that the redox, not the repair, function of APE1 may be involved in chemopreventive effect of oltipraz.

All of these compounds are natural agents that have been implicated in protecting against cancer.

B. Direct regulation of DNA repair by altered redox status of the cell

The evolutionarily conserved enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key redox-sensitive protein with an active-site cysteine sulfhydryl. GAPDH was shown to interact directly with APE1 (13). By using recombinant proteins, Azam *et al.* (13) found that GAPDH interacts with APE1 through the active-site cysteine 152 to convert the "oxidized" form of APE1 to its reduced form and reestablishes the ability to cleave AP sites. This reduction process also enhanced the detection of APE1 by anti-APE1 antibodies, suggesting a structural change. siRNA knockdown of GAPDH in HCT116 cells enhanced sensitivity to the alkylating DNA-damaging agent methyl methane sulfonate (MMS), which produces AP site, and increased the level of spontaneous AP sites in the genomic DNA. These data imply that GAPDH plays an important role in promoting BER activity by maintaining APE1, a key AP endonuclease, in an active reduced state.

OGG1, an 8-oxoG DNA glycosylase in the BER pathway, is responsible for recognizing and repairing 8-oxoguanine (8-oxoG), a common and mutagenic form of oxidized guanine in DNA. A recent study with human lymphoblastoid cells treated with cadmium resulted in an almost complete reduction in the 8-oxoG DNA glycosylase activity of OGG1, presumably because of an alteration in the redox status of the cells. OGG1 activity returned to normal once the redox cellular status was returned to normal. The reversible inactivation of OGG1 activity by cadmium was strictly associated with reversible oxidation of the protein, as demonstrated by the use of cysteine-modifying agents, such as diamide, with the pure protein and crude extracts (30). A frequently found polymorphism of OGG1, S326C OGG1, associated with cancer development, has been shown to have lower 8-oxoG DNA glycosylase activity, and the lower activity of OGG1-Cys326 is associated with the easy oxidation of Cys326 to form a disulfide bond (31). In this study, the 8-oxoG repair activity was analyzed in the cells and cell extracts of lymphoblastoid cell lines established from individuals carrying either Ser/Ser or Cys/Cys genotypes. The cells homozygous for the Cys variant display increased genetic instability, reduced 8-oxoG repair rates, and almost twofold lower basal 8-oxoG DNA glycosylase activity in their cell extracts. Reducing agents increase the repair capacity to the level of the Ser variant, but do not affect the activity of the latter. The 8-oxoG DNA glycosylase activity in cells carrying the Cys/Cys alleles is more sensitive to oxidizing agents (31).

NER, which repairs mainly bulky DNA adducts and helix-distorting lesions, has some crossover activity on oxidative DNA damage. On exposure of human pulmonary epithelial cells (A549) to nontoxic doses of H_2O_2, increased expression of *XPA*, *XPC*, *ERCC4*, and *ERCC5* was observed, whereas *ERCC1* expression decreased (114). Functional studies also demonstrated a decrease in NER activity.

Glutathione (GSH) also is directly implicated in the regulation of NER. GSH-depletion in cells preincubated with BSO, L-buthionine-sulfoximine, completely abolished the down-regulation of *ERCC1* expression and the decrease in NER capacity by H_2O_2 and increased significantly the upregulation of *ERCC4* expression. These data suggest that NER capacity as well as the expression of NER-related genes can be modulated by oxidative stress (114).

IX. Concluding Remarks

In conclusion, redox regulation clearly plays an important role in DNA repair, with implications for human health and cancer therapeutic development. We have highlighted the role of APE1, an important DNA-repair protein and redox factor, in this review as a protein directly linking redox regulation and DNA repair. It is our expectation that future research in this area will bring additional insights into the importance of redox regulation in DNA repair and will likely result in the identification of other proteins that also play important roles in redox regulation and DNA repair.

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Abbreviations Used

8-oxoG = 8-oxoguanine
AAG = alkyladenine DNA glycosylase
AGT = *O*-6-alkylguanine–DNA methyltransferase
Ang II = angiotensin II
AP = apurinic/apyrimidinic
AP-1 = activator protein 1
APE1 = apurinic/apyrimidinic endonuclease 1
ATF4 = activating transcription factor 4
ATM = ataxia-telangiectasia–mutated protein
BER = base-excision repair
BKca = large-conductance Ca^{2+}-activated K^+ channels
BLM = Bloom syndrome gene product
BRCA1 = breast cancer 1, early onset
BRCA2 = breast cancer 2, early onset
CD40L = CD40 ligand
CREB = cyclic AMP response element–binding protein
CSA = Cockayne syndrome protein A
CSB = Cockayne syndrome protein B
CTL = cytotoxic T lymphocyte
DDB1 = DNA damage binding protein 1
DDB2 = DNA damage binding protein 2
DNA-PK_{cs} = DNA-dependent kinase catalytic subunit
DR = direct repair
dRP = deoxyribophosphate
dRPase = deoxyribophosphodiesterase
DSBs = double-strand breaks
E3330 = 2E-3-[5-(2, 3 dimethoxy-6-methyl-1,4-benzoquinolyl)]-2-nonyl-2-propenoic acid
ERCC1 = excision-repair cross-complementation group 1
ERCC3 = excision-repair cross-complementation group 3
ERCC5 = excision-repair cross-complementation group 5
FEN1 = flap endonuclease 1
FPG = formamidopyrimidine DNA glycosylase
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
GGR = global genome repair
GRX = glutaredoxin
GSH = glutathione
GSSG = glutathione disulfide
HIF-1α = hypoxia-inducible factor-1
HR = homologous recombination
HR32B = human homologue of the yeast RAD23 protein
HUVECs = human umbilical vein endothelial cells
IL-2 = interleukin 2
LP-BER = long-patch base-excision repair
MeSeH = methylselenol
MGMT = O^6-methylguanine-DNA methyltransferase
MiTF = microphthalmia-associated transcription factor
MLH1 = MutL homologue 1
MMR = mismatch repair
MMS = methyl methane sulfonate
MPG = *N*-methylpurine DNA glycosylase
MRN = Mre11/Rad50/Nbs1
MSH2 = MutS homologue 2
MSH3 = MutS homologue 3
MSH6 = MutS homologue 6
MYH = MutY homologue
NAC = *N*-acetyl-L-cysteine
NEIL = fgp/nei family DNA glycosylase
NER = nucleotide-excision repair
NF-κB = nuclear factor kappa B
NHEJ = nonhomologous end joining
NK = natural killer
NTH = homologue of *Escherichia coli* endonuclease III (nth)
O^6-meG = O^6 methyl guanine
P2Y = purinergic receptor
PCNA = proliferating cell nuclear antigen
PMS1 = postmeiotic-segregation increased-1 protein
PMS2 = postmeiotic-segregation increased-2 protein
Pol β = DNA polymerase β
PRX = peroxiredoxin
PTEN = phosphatase and tensin homologue deleted on chromosome 10
Ref-1 = redox effector factor 1
RFC = replication factor C
RNA Pol II = RNA polymerase II
ROS = reactive oxygen species
RPA = replication protein A
SeMet = selenomethionine
siRNA = small interfering RNA
SMUG1 = mammalian 5-formyluracil DNA glycosylase
SP-BER = short-patch base-excision repair
ssDNA = single-strand DNA
TCR = transcription-coupled repair
TDG = thymine-DNA glycosylase
TFIIH = transcription factor IIH
TR = thioredoxin reductase
TRX = thioredoxin
TSH = thyroid-stimulating hormone
UNG = uracil-DNA glycosylase
WRN = Werner protein, deficient in Werner syndrome
XPB = xeroderma pigmentosum complementary group B protein
XPC = xeroderma pigmentosum complementary group C protein
XPD = xeroderma pigmentosum complementary group D protein
XRCC1 = x-ray repair complementing defective repair in Chinese hamster cells 1
XRCC4 = x-ray repair complementing defective repair in Chinese hamster cells 4